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BIOLOGICAL BULLETIN

THE RELATIVE PERMEABILITY OF THE SURFACE AND INTERIOR PORTIONS OF THE CYTOPLASM OF ANIMAL AND PLANT CELLS.[1]

(A Preliminary Paper.)

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The permeability and osmotic properties of cells are explained on the assumption of the presence of surface and vacuolar plasmatic membranes which are of a semi-permeable or partially permeable character. It is generally held that the remainder of the protoplasm of a given cell plays a negligible rôle in both diosmosis and permeability. These assumptions are based on indirect evidence. The permeability of the internal cytoplasm of living animal and plant cells has not been investigated by direct methods.

This paper gives the results of a study of the permeability of the internal cytoplasm and nucleus to dyes and crystalloids.

The animal material used in this investigation included the eggs of *Asterias*, *Cumingia*, *Chætopterus*, *Nereis* and the immature eggs of *Necturus*, *Ameba proteus*, *Paramecium* and the striped muscle and epidermal cells of *Necturus*. The plants selected were *Saccharomyces*, *Mucor*, *Saprolegnia*, some five species of *Spirogyra*, *Hydrodictyon*, the manubrial cells of *Chara*, the leaves of *Elodea*, root-hairs of *Vicia faba*, *Pisum*, *Hordeum* and the parenchyma cells of *Tradescantia*.

[1] The studies on marine eggs reported in this paper were carried on during the summer of 1912 at the Marine Biological Laboratory, Woods Hole, Mass., while occupying a table through the courtesy of the Director, Dr. F. R. Lillie, to whom I am indebted for many kindnesses.

A number of methods were used in order to make the data comparative and to determine the error and limitations of a given method employed. The effect of various operations, such as partial dissections, and punctures, on the permeability and osmotic properties of cells was studied. Intracellular injections were made by Barber's method.[1]

The Permeability of Animal and Plant Cells to Acid Dyes.

Incidental to this study, a number of facts bearing on Overton's so-called lipoid theory have been determined, and as animal physiologists have taken kindly to this notion some of these data will be given.

Rhuland[2] and others have shown a number of exceptions to Overton's[3] conclusion that cells are impermeable to lipoid insoluble acid dyes.

By using a large number of species of animals and plants from widely separated genera and phyla, I have found it quite easy to discover cells that are freely permeable to many lipoid-insoluble acid dyes. Such well-known acid dyes as eosin and trypan red are good vital stains for *Mucor* and *Saprolegnia*.

The lipoid-insoluble acid dyes used include:

Trypan blue, which penetrates the eggs of *Nereis* and *Chætopterus*, the root-hairs of barley and the Windsor bean, immature *Necturus* eggs, the peritoneal epithelium of *Necturus*, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Trypan red: the eggs of *Cumingia* and *Chætopterus*, the root-hairs of barley, the edible pea and the Windsor bean, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Isamin blue: the root-hairs of barley and the Windsor bean.

Analine blue: the eggs of *Chætopterus*, the root-hairs of barley and the Windsor bean, and *Mucor*.

Acid fuchsin: The root-hairs of barley, the Windsor bean and *Mucor*.

Acid green: *Saprolegnia* and *Mucor*.

[1] Barber, *Jour. of Inf. Dis.*, 1911, VIII., p. 348.
[2] *Jahr. Wiss. Bot.*, Bd. 51, H. 3, p. 376.
[3] *Jahr. f. Wiss. Bot.*, Bd. 34, 1900, p. 669.

Acid violet: Windsor bean root-hairs, *Saprolegnia*, and *Mucor*.

Beibricher scarlet: Barley root-hairs, the immature eggs of *Necturus*, the peritoneal epithelium of *Necturus*, *Mucor*, *Saprolegnia*, and *Paramecium*.

Indigo-carmin: *Saprolegnia* and *Mucor*.

Ponceau, P. R.: the root-hairs of the Windsor bean, *Ameba proteus*, *Paramecium*, *Saprolegnia*, and *Mucor*.

Indulin: root-hairs of Windsor bean, *Ameba proteus*, *Saprolegnia*, and *Mucor*.

Nigrosin: the peritoneal epithelium and the very immature eggs of *Necturus*, and *Mucor*.

Eosin: Windsor bean root-hairs, *Ameba proteus*, *Paramecium Mucor*, and *Saprolegnia*.

In recent papers Loewe[1] states that even basic dyes are adsorbed by lipoids, when added to solutions of lipoids in organic solvents.

THE INTRACELLULAR INJECTION OF DYES AND CRYSTALLOIDS.

If indigo-carmin, methyl red, trypan blue, thiocarmine R., or azolitmin dissolved in sea-water be injected into any portion of the cytoplasm of the starfish egg, the sea water slowly diffuses into the surrounding protoplasmic gel, and finally the granular precipitated dye alone remains. An injection of indigo-carmin into the cytoplasm of an immature egg of *Necturus* results in a slight staining of the wall of the vacuole, while an injection of such widely different dyes, as indigo-carmin, trypan blue, and janus green (diethyl-safranin-azo-dimethyl-anilin), into the cytoplasm of the striped muscle cell of this animal results at most in a localized staining of the cytoplasmic gel immediately surrounding the mass of injected dye. The injection of indigo-carmin or nigrosin into the vacuole of *Spirogyra* results in a blue-green or light violet staining, respectively, of the entire cytoplasm. A blue-green staining of the cytoplasm of *Hydrodictyon* is affected by an intravacuolar injection of indigo-carmin. In fact, every acid dye that was injected into the vacuole of *Spirogyra*, *Hydrodictyon*, the leaf cells of *Elodea*, and the parenchyma cells of *Tradescantia* penetrated, and stained the cytoplasm and usually the nucleus.

[1] Loewe, *Biochemische Zeitschrift*, 1912, XLII., p. 150.

Particularly striking permeability phenomena are to be observed when dyes that do not penetrate *Ameba proteus* are injected into the interior. Azolitmin, congo red, tropeolin ooo No. 1, sodium alizarin sulphonate, and indigo-carmin were used for these injections.

All the dyes enumerated were dissolved in salt and sugar solutions of different concentrations, and small and large doses were injected. It was found that these dyes diffuse quickly through the interior of *A. proteus*, if the concentration of the salt or sugar solution be not too high. A localized blue vacuole results when indigo-carmin, dissolved in distilled water, is injected into the ectoplasm of this animal. Usually in a short time the vacuole breaks into the interior and the dye rapidly diffuses.

Sea water, distilled water, solutions of sodium chloride, potassium nitrate, and cane sugar have been injected into the interior of different types of cells. A small dose of distilled water is taken up by the surrounding cytoplasm of the starfish egg quite slowly. A vacuole of sea water requires a somewhat longer time to disappear, while a vacuole filled with hypertonic sea water increases in size. Hence, any portion of the cytoplasm of the starfish egg can exhibit the same general diosmotic properties that are shown by the surface. It seems that this is also true for the cytoplasm of the striped muscle cell of *Necturus*, but on account of the very high viscosity of this substance the wall surrounding the injected fluid remains very irregular, and no accurate measurement of the volume injected could be made. Distilled water is absorbed from the interior of the muscle substance extremely slowly; and salt and sugar solutions more slowly or not at all.

Doses of 1 m. sodium chloride and potassium nitrate and from .5 M. to 2 M. cane sugar diffuse through the cytoplasm of *Ameba proteus* when injected into the interior. The vacuoles that are formed by the injected fluid quickly collapse. Granules, fibrils, and globules can be produced in *proteus* by the injection of 2 M. cane sugar. The shrunken part of the cytoplasm does not readily take up water again.

In this connection it may be added that the injection of mercury and isotonic salt solutions and the introduction of various

foreign bodies into protoplasm usually lead to remarkably little reaction.

The Effect of Chemical Treatment and Operations on the Permeability of Cells.

Early in this investigation a general relation between permeability and the degree of concentration of protoplasmic gels was noted. Several methods were devised for grading the concentration of the surface and interior portions of the cytoplasm of various cells. The desired concentration gradient can be produced in the eggs of *Asterias*, *Chætopterus*, *Cumingia*, and *Nereis* either by treating with very dilute acids, alkalies and saponin dissolved in sea water or puncturing or cutting with extremely fine Jena glass needles. It was proved that such operative treatment does not kill any portion of the egg.

If the egg of *Asterias* be punctured, the acid dyes used penetrate the swollen area for varying depths, but never enter the normal unswollen cytoplasm. Under the conditions of my experiments only the swollen surface was penetrated and stained. It cannot be overemphasized that the concentration gradient experiment has proved an adequate test of the correctness of the membrane conception, at least for the eggs of *Asterias*, *Cumingia*, *Chætopterus* and *Nereis*. Dyes were selected which do not penetrate the normal egg. These dyes penetrate the swollen cytoplasm produced by the operation or chemical treatment, to varying depths, but never enter the unswollen cytoplasm. Some of the acid dyes do not penetrate the surface of the swollen area of a punctured egg, while others are stopped only by the unchanged cytoplasm. These results are exactly the converse of what would necessarily follow if the plasmatic membrane conception were really true. Moreover, it was noted that trypan red and erythrosin stain the surface of a normal *Chætopterus* egg, but even a great increase in concentration of the dyes does not produce more than a surface staining. Here the surface is actually more permeable to these dyes than the interior of the egg. Again, the nucleus of the eggs of *Asterias*, *Cumingia*, *Chætopterus*, and *Nereis* remain unstained when the eggs are placed in the best vital stains in use at the present time. Under this set of condi-

tions the nuclear membrane is impermeable to vital stains. This structure is a concentrated tough gel of relatively high viscosity and is not to be confused with hypothetical surface or vacuolar plasmatic membranes. If the concentration of a suitable vital stain be raised considerably or the nucleus be dissected out, staining occurs.

Puncture of the walls and probably the cytoplasm of various types of plant cells has given unanticipated results. All the more common acid dyes enter the cells of such plants as *Spirogyra*, *Elodea*, *Hydrodictyon*, different root-hairs, and the parenchyma cells of *Tradescantia*, following an extremely small puncture. Puncture of the walls of slightly plasmolyzed *Spirogyra* and *Elodea* cells is followed by protoplasmic staining when acid dyes are added. On the other hand, puncture of thoroughly plasmolyzed *Spirogyra* cells is followed at most by a slight surface staining, of the shrunken and concentrated cytoplasm, by the same acid dyes.

During plasmolysis of *Spirogyra* a large amount of mucilaginous material is poured out of its wall, and, as a result, this structure loses much of its rigidity, becomes softer, and permeable to many acid dyes. This striking change can be demonstrated by vital stains, dissection of the wall of thoroughly plasmolyzed cells, and by staining cells immediately after recovery from thorough plasmolysis.

A very small cut in the wall of a manubrial cell of *Chara* gives even with trypan blue and trypan red only a local staining of the cytoplasm, which slowly spreads.

The Dissection of Cells.

The chief cells that have formed the material for this study have been dissected by the use of adequate methods, and their physical properties, such as rigidity, viscosity, glutinicity, elasticity, tenacity, and colloidal state, have been determined. Although these data are too extensive to be given here, they form a part of the basis of my conclusions.

Conclusions.

1. The structural components of protoplasm vary greatly in their permeability to water, dyes, and crystalloids.

2. Impermeability or partial permeability to water, dyes, and crystalloids is a property of all portions of protoplasmic gels.

3. The rate of penetration of protoplasm by dyes and crystalloids is, in general, inversely proportional to the concentration of the living gel.

4. The best vital stains known penetrate such highly concentrated protoplasm as the epithelial and striped muscle cells of *Necturus* very slowly.

5. The interior portions of the cytoplasm of the starfish egg and probably the striped muscle cell of *Necturus* exhibit the same sort of osmotic properties as the surface.

6. The cell-walls, and not the protoplasm of many plant cells, prevent the entrance of dyes.

SPERMATOGENESIS OF THE PIG WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOMES.

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INTRODUCTION.

In late years the occurrence of accessory chromosomes, in various degrees of complexity, has been recorded from diverse groups of invertebrates. Recently the field has been extended to the vertebrates, Guyer having shown their existence in the males of the guinea ('09*a*), rooster ('09*b*), rat ('10), and man ('10); Newman and Patterson ('10) in the armadillo; Jordan ('11) in the opossum; Stevens ('11) in the guinea-pig; and King ('12) in *Necturus*. The existence of what appears to be such an element has also been reported in the ovary of the cat by Winiwarter and Saintmont ('09). In a recent paper Jordan ('13) states that the heterochromosomes are unquestionably lacking in the pig. I find, however, that the accessory chromosomes are very conspicuous in all of the pig material which I have studied.

Wilson ('09) found in *Syromastes* that half of the spermatids possess two more chromosomes than the remainder. It was predicted by him that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male, and later the facts were found to be in exact accord with his predictions, the somatic cells of the female of *Syromastes* having been found to contain twenty-four, those of the male, twenty-two chromosomes. A similar condition has been found in other tracheates, but dimorphism in the number of chromosomes in the germinal and somatic cells of the two sexes among the vertebrates has thus far been only inferred, not actually demonstrated.

The present study on the spermatogenesis of the pig was taken up at the suggestion of Professor M. F. Guyer, to whom I am much indebted for many helpful suggestions during the progress of the work. Points of special interest in this paper are as follows:

1. The presence of a distinct pair of accessory chromosomes.
2. The resulting dimorphic condition in the spermatozoa of the pig.
3. Dimorphism in the number of chromosomes in both the germinal and somatic cells of the male and female animals.
4. The abundance of large conspicuous interstitial cells in the testes.
5. The second reduction of the chromosome number in the secondary spermatocyte, which was found to be simply equational.
6. The throwing off of a large mass of cytoplasm, containing one of the centrosomes, at the time of the final development of the spermatozoan.

Material and Methods.

I have been very fortunate in obtaining from several sources exceptionally good material for this investigation. The major part of the material studied was obtained from a vigorous Poland China boar about ten months old. The animal came from registered stock and was the property of the College of Agriculture of the University of Wisconsin. The material was ob-

tained through the courtesy of Professor L. J. Cole. Immediately after the testes were removed from the live animal, pieces were placed in various kinds of fixing fluids, as Bouin's, Zenker's, Tellyesnicky's, and corrosive-acetic.

Sections from various parts of the testes were made from four to twelve microns thick and while several methods of staining were employed, material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, proved to be the most satisfactory. Delafield's hematoxylin with eosin counterstain was also used and gave favorable results. While material fixed in some of the other fluids mentioned was fairly good, the first method proved to be so superior that the sections used in this study were almost wholly those prepared according to it. Other material, from a much older animal, was studied in sufficient detail to corroborate the results obtained through the study of the material secured from the younger and more vigorous animal.

I also owe many thanks to Professor B. M. Allen for his generosity in placing at my disposal his embryological material of pigs of both sexes. The material in question, which was that used by Dr. Allen ('04) in his own researches on the embryology and development of the ovary and testes in mammals, was fixed in Flemming's fluid and stained with Heidenhain's iron hematoxylin mainly. By examining a large number of these slides, I not only corroborated my previous count of the spermatogonial number of chromosomes, but was also able to make fairly conclusive counts of the chromosomes in the somatic cells of the male, and in the oögonia and somatic cells of the female. Without the use of his material this important phase of the problem could not have been worked out at this time.

General Arrangement of the Germinal Cells.

The structure of the testes of the pig does not differ greatly from that of other well-known mammals, except for the presence of numerous masses of large well-defined interstitial cells which are scattered among the seminiferous tubules and comprise about one fourth of the entire volume of the testes (Fig. 1). A detailed description of these interesting cells, which contain

numerous mitochrondia, will be reserved for a separate paper. The germinal cells themselves are found in a great number of seminiferous tubules coiled throughout the interior of the testes. Sections of the tubules are found in groups of fifteen to twenty-five which are completely surrounded by walls of connective tissue. This connective tissue forms a sort of continuous network of walls throughout the testes and is rather thick in places, usually where three or four of the groups become appressed. Embedded in these walls are blood- and lymph-vessels which branch out into the masses of interstitial cells between the tubules.

The arrangement of the cells in the tubules is similar to that of the other warm-blooded animals and the usual four types of germinal cells are present. The spermatogonia form a more or less regular layer of cells lying next to the wall of the tubule. These divide and give rise to two new cells, one or both of which may become the primary spermatocyte. The primary spermatocytes are very abundant, especially in the spireme stage. After various changes and considerable growth the primary spermatocytes divide and give rise to the secondary spermatocytes. The secondary spermatocytes divide in turn to form the spermatids, which transform directly into spermatozoa (Figs. 64–78).

The material studied must have been in maximum activity at the time of fixation, for the four types of germinal cells were easily found in stages of growth and development. Mitotic stages of spermatogonia, primary and secondary spermatocytes, were very abundant, and frequently the entire field under the oil immersion lens was composed of cells in mitosis.

Spermatozoa in all stages of development were very abundant, and clusters of the cells which were in the final stages of development could be seen attached to the long cylindrical Sertoli cells which often extend to the lumen of the tubule (Figs. 2 and 3). The Sertoli cells, which are quite abundant, are closely connected with the germinal cells and will be described in more detail later (Fig. 63).

Spermatogonia.

The spermatogonia usually lie in a single layer next to the wall of the tubule though occasionally some of the cells are

crowded out, thus forming a second layer which is always very irregular. There is considerable variation in the appearance and some variation in the size of different spermatogonia. Very frequently the cells are far apart, in which case they are flattened out on the tubule wall.

The amount of cytoplasm is small and in some of the earlier stages the cell boundaries are very indistinct (Fig. 16). Later the nuclei assume a round shape and the cell wall becomes visible. The amount of chromatin material increases and the nuclei at this stage resemble very much the nuclei of the large interstitial cells both in appearance and size (Fig. 17).

Two large nucleoli much alike in size seem to be present in all stages of these cells. Even in the testes of young embryos the two bodies are very conspicuous. Besides these large nucleoli a varied number of smaller, similarly staining bodies are usually present, two of which seem to occur most frequently, and evidently make their appearance first (Figs. 16 and 17). Later other small nucleoli appear. At present I am unable to attach any meaning to them as their numbers are so varied in the various cells. The two large nucleoli, however, are very constant, and I have been able to trace them throughout the entire spermatogenesis. From all appearances one is led to believe that these nucleoli and the accessory chromosomes are one and the same, a condition similar to that found by Guyer ('10) in man, and by others in some of the lower forms. Jordan ('11), however, has not been able to identify any such structures at this stage as the future accessory chromosomes in the opossum.

At the conclusion of the resting stage numerous chromatin granules appear, which arrange themselves along fine threads in an entangled mass. The two nucleoli come in close contact and the nuclear membrane gradually disintegrates. Eighteen chromosomes appear in the late prophase of the spermatogonial division (Fig. 18). Sixteen of these are rod-shaped, variously curved and somewhat different in size. Two, which are always found close together, and frequently off to one side are slightly larger and oval in shape. The sixteen ordinary chromosomse or autosomes arrange themselves with the two accessory chromosomes in the equatorial plate (Fig. 19). The accessories, like

the autosomes, divide in this stage. A centrosphere containing a tiny centrosome can be observed in some cases, but its presence is not at discernible in these early cells as it is in the later stages.

Primary Spermatocytes.

1. *Resting Stage.*

The primary spermatocytes which arise from the final spermatogonial division in the early resting stage are usually somewhat smaller than the spermatogonia in the metaphase and prophase stages. The two large nucleoli are quite conspicuous and remain black in sections stained with iron hematoxylin even in very much destained material (Fig. 20). Usually two small nucleoli, stained the same way as the large ones, are plainly visible and occasionally only one, or more than two of these bodies appear.

2. *Synizesis and Growth Period.*

An increase in the bulk of both the nucleus and cytoplasm begins after a brief period of rest. The chromatin appears to be arranged in much the same way as it was during the resting condition of the spermatogonia, except that the linin fibers are coarser. The cytoplasm is composed of granular masses and clear, apparently liquid areas. The chromatin threads and nucleoli become massed at one side of the nucleus (Fig. 21). The nuclear wall expands and the clear area formed by the massing of the chromatin to one side becomes much enlarged. The clear areas in the cytoplasm decrease coincidentally with the increase of the liquid-like area in the nucleus and one is led to believe that the cytoplasmic fluid permeates the nuclear wall during the period of synizesis.

During the collapse of the chromatin material, the nucleoli can be plainly seen especially in well destained sections (Fig. 21). They are usually in that portion of the chromatin mass which is nearest to the nuclear wall. Opposite this mass on the outside of the nuclear wall a centrosphere can often be seen, but the centrosome is rarely visible at this stage. The chromatin threads become arranged in a very much tangled mass of loops which later appear in about half the original number and fully twice as thick. (I have not been able to determine definitely whether

the pairing takes place by telo- or parasynapsis.) The whole mass then moves toward the center (Fig. 22), the large clear area in the nucleus disappears, and the nuclear wall becomes spherical and clearly defined (Fig. 23). While the formation of the spireme is going on, the cytoplasm, too, increases greatly in volume and appears very granular. The cell now is fully twice as large as a spermatogonium and judging by the large number of cells that can be seen in the spireme stage one can safely conclude that they remain in that condition for some time. The spireme finally breaks up into U and variously shaped chromosomes (Fig. 25). The two large nucleoli, which remain in full view throughout this stage, become oblong and can often be seen close together (Fig. 25).

3. *Reduction Division.*

The primary spermatocytes when ready for division reveal ten chromosomes in the late prophase or early metaphase stage. The two accessories which are ordinarily off to one side (Figs. 8, 26, 27, and 29) can be recognized at a glance. The other chromosomes are usually arranged in a ring. Judging from their large size and changed form, they are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original eighteen chromosomes, sixteen have paired to form eight bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. Sometimes, although ten chromosomes can be counted, it is difficult to tell just which are the accessories owing to overlapping. The chromosomes differ somewhat in size as can be seen in Figs. 26 to 29. Figs. 9 to 12 and 30 to 37 show the accessories in characteristic positions in the metaphases of division of the primary spermatocyte. They always pass entire, side by side and in advance of the divided autosomes, toward one pole. This is possibly due to the fact that they are not retarded by division.

The fact that the chromosomes immediately after divergence (Fig. 28) resume the appearance (except in size) that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole in this division while they are halved in the next division, seems to indicate strongly that this is the reduction division.

It is obvious that as far as chromatin content is concerned the division of the primary spermatocyte gives rise to two dissimilar cells, one of which receives eight chromosomes, and the other eight plus the two accessories or ten chromosomes (Fig. 38). Figure 39 is a drawing of one end of a late anaphase of such a division showing eight chromosomes, and Fig. 40 shows the other end which received eight of the ordinary chromosomes and the double accessory.

Occasionally a small chromatin body is present in this first spermatocytic division (Figs. 28, 31, 32, 35 and 37). Figure 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Figure 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. Figure 28 shows the body outside of the main ring of chromosomes. While the small body can be seen frequently, as a rule no such an element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance.

Secondary Spermatocyte.

1. *Dimorphism.*

The dimorphism of the secondary spermatocytes, which resulted from the last division, is again expressed in the resting stage that sometimes follows. Approximately half of them showed, under proper decolorization, two large chromatin nucleoli (Fig. 41) while in the others only the small nucleoli appeared (Fig. 42). The nucleoli retain the usual deep staining capacity, and as was true in the previous stages, even when all the other material is almost totally decolorized, the nucleoli remain very conspicuous. Frequently, both primary and secondary spermatocytes were found dividing in the same field, which fact seems to suggest that at times there is no intervening period of rest between the two divisions, or that it is very brief. Figure 43

shows the two resulting cells of a primary spermatocyte division which are still in close contact and both ready for dividing into spermatids. Resting stages, however, appear in abundance and in this stage, as was the case in the resting stage of the primary spermatocyte, the centrosome surrounded by a clear zone becomes large and very conspicuous (Figs. 41 and 42).

2. *Second Reduction Division (Equational).*

Although the division of the primary spermatocyte gave rise to cells containing eight and ten chromosomes respectively (Figs 38, 39, 40), when these cells become ready for division half of them show four (Figs. 13, 14, 43, 45, 46) and the other half six chromosomes (Figs. 43, 44, 47). Thus a second pairing of the ordinary chromosomes, similar to that found by Guyer in the pigeon ('00), corroborated by Geoffrey Smith ('12), man ('10), guinea ('09*a*), and chicken ('09*b*), and by Jordan in opossum ('11), has evidently taken place so that there are four bivalents in each type of cell and the additional two accessory chromosomes in the one type. Stevens ('11) says that there is no such second synapsis or numerical reduction in the guinea-pig. Figures 45 and 46 show four large chromosomes in the metaphase stage of one type of secondary spermatocyte and Figs. 44 and 47 represent the other type in which six chromosomes appear, four bivalent autosomes plus the two accessories. The four chromosome group is evidently formed by the pairing of the eight chromosomes of one type of cell resulting from the first maturation division at one pole, and six chromosome group is interpreted as being derived by the pairing of the eight chromosomes plus the two unpaired accessories at the opposite pole (Figs. 38, 39, 40).

Guyer ('10) in speaking of the second conjugation of the chromosomes in man says:

"Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia, or in ana-

phases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that is characteristic of the bivalent types (compare Figs. 1, 10, and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively."

Jordan ('11) in speaking of the same condition in the opossum remarks as follows:

"Similarity of form between the chromosomes of the first and second metaphase plates (*i. e.*, double rods) suggests a similar manner of division: accordingly a second reduction. When one recalls, however, that a resting stage (Figs. 39 to 41) usually intervenes between the first and second maturation divisions, when the chromosomes pass through a reticular phase, the above conclusion is inadmissible; or rather, no definite conclusion respecting the character of the second division is justified. A double true reduction, suggested by the form of the chromosomes is contrary to our fundamental conceptions regarding the significance of chromosomes and need not, in view of the nature of the evidence, be seriously considered. Moreover, in the stage just preceding the brief resting phase of the spermatids (Figs. 57, 58 and 59) there occurs a resolution of the five chromosomes into nine and of the four into eight. This demonstrates that the true character of the second division is equational. The second numerical reduction involves a less close union apparently than the first, as a comparison of illustrations 29 and 43 will show. Again the fusion is sometimes incomplete to the extent of giving an occasional count of six chromosomes."

My own belief that the real character of the second division in the case of the pig is simply equational is based on a number of facts. Even after the first maturation division the ordinary chromosomes are much larger than the spermatogonial univalents. This increase in size has evidently taken place during the growth period of the primary spermatocyte (compare Figs. 18 and 38). During the prophase of the secondary spermatocyte the chromosomes apparently increase still more in size and four large bivalent autosomes appear for division in the one type of cell and

four plus the two accessories in the other type. Not only is the bivalent nature of these large autosomes conspicuous, but very frequently a quadrivalent character is discernible and they are much larger than the accessories (Figs. 44, 45, 46 and 47). During the anaphase the bivalent nature of the chromosomes is often clearly visible at each pole. Figure 49 shows four bivalents and is a drawing of one pole of the dividing cell which received eight chromosomes during the first maturation division. Figure 52 is a drawing of one pole of the division of the type of secondary spermatocyte which received eight ordinary chromosomes and the double accessory. It will be seen that four of these are bivalent in nature, while two are univalent, the two univalents being the results of the division of the two accessories (Figs. 44, 50 and 51) both of which have here divided for the first time since the spermatogonial division. The bivalent nature of the autosomes after the second spermatocytic division becomes even more conspicuous in cases where these chromosomes divide into two before they break up. Figure 56 shows an early spermatid cell which received the four bivalent autosomes and the two accessories. It can be seen that two of the autosomes have almost completely divided. The bivalent nature of the other two can also be plainly seen, while the accessories retain their univalent appearance. Thus it can be seen that the spermatids produced by this second division do not receive four and six chromosomes respectively, but, four bivalents, or the equivalent of eight univalents, are present in the one type of cell, and four bivalents or eight univalents together with the two accessories in the other. The foregoing facts seem to indicate beyond doubt that the second maturation division is not a reduction but simply an equational one.

Figures 50 and 54 represent an anaphase of division in a secondary spermatocyte showing two streaks of lagging chromatic material. Although several such cases were observed ordinarily no such pronounced streaks occur (Fig. 51). Guyer found a similar condition in the secondary spermatocyte division in man and suggests that it may be the two accessories lagging behind. Although many accurate counts of the chromosomes in this second division were made, they were so massed together

as to render a count impossible (Fig. 54). Not infrequently the chromosomes in the early spermatids become closely appressed before they begin to break up (Figs. 53 and 57). Sometimes the individuality of the chromosomes is apparent even after the nuclear wall begins to form (Fig. 58).

Spermatids.

From the foregoing evidence it is obvious that there exists a dimorphism among the spermatids, one type containing eight and the other ten chromosomes after the last division. The chromosomes soon become irregular in shape and begin to break up, and the spermatids appear all alike except that immediately after the chromosomes disintegrate two nucleoli are visible in approximately half of the cells (Figs. 64 and 65). These retain for a short time the staining capacity characteristic of the nucleoli of the previous stages, but later disappear and the whole nucleus assumes a coarse granular appearance. The cells seemingly remain quiescent in that condition for some time as they are nearly always found in large numbers. The centrosome surrounded by a clear layer is again very conspicuous within the comparatively small centrosphere which lies close to the nucleus (Figs. 64 and 65).

Development of the Spermatozoa.

Following the period of rest the spermatids begin to develop into spermatozoa. There is no perceptible change in the size of any of the parts, such as described by Jordan in the opossum. The first change to be detected is the extrusion of the centrosome, surrounded by a light area, from the small sphere (Fig. 66). It takes a position a short distance from the nucleus and soon begins to divide. The two new centrosomes move apart and one, somewhat rod-shaped, comes in contact with the nuclear wall but it remains connected by a mass of material with the other centrosome which assumes a disk shape and for a while remains in place (Figs. 67, 68 and 69). The disk is frequently perforated in the middle and has the appearance of a ring (Fig. 70).

Simultaneously with the division of the centrosome, a portion of the sphere which remains in close contact with the nuclear wall

migrates to a point directly opposite the centrosome in contact with the wall on the other side (Figs. 66, 67 and 68). The sphere in this process of migration is in such close contact with the nuclear wall that a slight depression can be detected in the latter. The depression is even more pronounced when the clear sphere becomes definitely fixed as the small acrosome (Figs. 68–75).

In the guinea-pig the acrosome, which becomes nearly as large as the nucleus itself, according to Meves ('99), is also formed from the centrosphere or idiozome. While in the rat, according to Lenhossek, it is independently formed in the cytoplasm without relation to the preceding mitotic figure or the centrosomes. Meves ('97) also found that in the salamander the acrosome is formed from the idiozome which wanders around the nucleus to its anterior pole. McGregor's results on *Amphiuma* ('99) agree in general with those of Meves, except that here the acrosome arises from only a part of the centrosphere, while a second smaller part passes to the base of the nucleus and forms the main part of the middle-piece. Coincidentally with the division of the centrosome and the migration of the small sphere the nucleus together with these structures moves to one side of the cell in the direction of the acrosome and soon practically all of the cytoplasm is at the posterior end of the cell (Figs. 66, 67, 68, 69 and 70). The cell wall seems to persist as a thin mantle covering the head of the spermatozoan.

The anterior part of the cell, bearing the acrosome, almost invariably points in the direction of the tubule wall while the mass of cytoplasm extends into the lumen (Fig. 2). Most of the chromatic material of the nucleus gathers at the center into a dense mass which has the same staining capacity as characterizes the chromosomes and the centrosome. Sometimes two or three masses are present (Figs. 73, 74 and 75).

Soon after the division of the centrosome into a cylindrical anterior and a disc-shaped posterior body, a divergence of the two follows, but they remain connected by a streak of material (Fig. 69). The inner body upon coming in contact with the nuclear wall forms a depression in the latter and its anterior portion shapes into a disc which comes in close contact with the nuclear wall in the small depression at the posterior end of the

nucleus. From this disc, which is apparently the end-knob, a cylindrical mass of material extends backwards uniting the two centrosomes (Figs. 68 and 69). A tiny filament extending backward from the middle of the posterior disc-shaped centrosome makes its appearance and is undoubtedly a continuation of the coarser filament which unites the two centrosomes (Fig. 69). As the tail grows longer the connecting filament becomes thinner, and the posterior centrosome, which after division was disc-shaped and had increased somewhat in size, becomes transformed into a ring and it can be seen that the filament extends directly through it and continues backward as the tail (Fig. 70). The formation of the ring takes place simultaneously with the rapid growth of the tail and one is led to believe that the perforation in the disc is partly due to the fact that the material formerly occupying that space goes to help in building up the axial filament. Shortly after the tail projects out of the cell the ring moves along the filament, backward, and soon swerves over to one side in the cytoplasmic mass (Figs. 71 and 72). Very frequently it can be seen a considerable distance away from the axial filament long before the latter is fully developed (Figs. 73, 74 and 75). This seems to indicate that the posterior centrosome takes no further part in the development of the filament and that the latter is mainly developed from the anterior centrosome, no part of which is discarded or thrown off. While most of the inner centrosome passes into the formation of the axial filament a part of it remains as the end-knob in the small middle-piece (Figs. 76 and 78), a condition similar to that found by McGregor in *Amphiuma* ('99).

The posterior ring-shaped centrosome, after moving away from the filament, sometimes divides (Fig. 73), but usually assumes a spherical shape (Fig. 74) and an interesting point in connection with this body is that during the final development of the spermatozoan it is invariably thrown off with a big mass of cytoplasm (Figs. 74, 75 and 76). The casting off of a portion of the cytoplasm during the last stages of the developing spermatozoan has been described by Meves ('99) in the guinea-pig where it is closely similar to the process which occurs in the spermatozoid-formation in ferns; but the throwing off of a portion of the

centrosome together with the mass of cytoplasm has not heretofore been, to my knowledge, recorded. The body usually stains as deeply as it does in the earlier stages for some time after the comparatively large mass of cytoplasm containing it is completely separated from the rest of the cell or young spermatozoan.

In general, the behavior of the centrosomes in the development of the spermatozoan of the pig does not differ greatly from the conditions found in some of the other vertebrates. However, it might be well to briefly point out some of the differences. In the spermatids of the salamander, according to Meves ('97), the two centrosomes lie quite at the periphery of the cell and from the outer one grows out the axial filament. The two centrosomes leaving the idiozome by which they are first surrounded, now pass inwards toward the nucleus, the outer one meanwhile becoming transformed into a ring while the axial filament passes through it to become attached to the inner centrosome. The latter pushes into the base of the nucleus and enlarges enormously to form a cylindrical body comprising the main portion of the middle-piece. The ring divides into two parts, the anterior of which gives rise to a small body at the posterior end of the middle-piece identical with the end-knob. The other part of the ring wanders out along the tail and finally lies at the limit between the main part of the latter and the end-piece.

In the pigeon, according to Guyer ('00), the centrosome divides and moves out of the sphere and further away from the nucleus. The two new centrosomes move apart, but remain connected by a mass of material which later disappears and a very delicate fibril uniting the two centrosomes exists in its place. One of the centrosomes enlarges and transforms into a complete ring. The connecting fibril can later be seen passing from the smaller centrosome back through the ring and outside the cell. The centrosomes next approach the nucleus and as they draw near a slight invagination appears in the nuclear wall and the small centrosome moves into it.

In the mammals, the work of Lenhossek on the rat ('98) and Meves on the rat, guinea-pig and man ('98, '99) gives a result similar to the condition found in the salamander. In all these mammals the young spermatids contain two peripherally placed

centrosomes, from the outer one of which the axial filament grows out and the centrosomes later move toward the nucleus. In the pig the centrosomes are never peripherally located and division of the spermatid centrosome does not take place until it emerges, surrounded by a small clear sphere, from the main bulk of the centrosphere which is in close contact with the nucleus.

It can be seen from the drawings of the later stages that the nucleus gradually elongates and flattens (Figs. 67–78). The large chromatic mass within the nucleus disintegrates and the particles become distributed at the periphery of the nuclear wall (Fig. 78). The tail envelope becomes apparent as the cell elongates and evidently develops from the cytoplasm (Figs. 74–78). It later comes in contact with the axial filament and envelops about half of its entire length. The remaining extremely thin portion of the tail is apparently the naked axial filament (Figs. 76, 77, 78).

When the cells reach the stage represented in Figs. 71–73 they attach themselves in bunches to the large cylindrical Sertoli or nurse cells that often extend from the basement membrane a long distance toward the lumen of the tubule (Figs. 2 and 63). As the spermatozoa continue to develop a gradual decrease in the volume of the cytoplasmic mass of the Sertoli cells is noticeable. When the spermatozoa are apparently mature they abandon the Sertoli cells which at this time are very much collapsed. The spermatozoa after leaving the nurse cells do not pass directly to the lumen of the tubule, but remain scattered for some time among the masses of cytoplasm which were cast off by the same developing cells a short time before (Figs. 3 and 67). This cast-off material does not scatter about in the lumen, as one would suspect, but forms a sort of loose layer next to the cells following the maturing spermatozoa. The masses begin to disintegrate soon after they are discarded by the developing spermatozoan, and when stained with iron-hæmatoxylin numerous black bodies make their appearance in this material (Fig. 3). The spermatozoa remain, with their bodies embedded in this layer, and tails extending into the lumen, until the mass almost completely disappears. Then they become free in lumen of the tubule and are ready to make their way out of the testes. During this period

of attachment to the nurse cells and later to the discarded mass of cytoplasm the heads of the spermatozoa increase somewhat in size but retain the same staining capacity. The foregoing facts seem to show beyond doubt that the developing spermatozoan derives nourishment not only from the nurse cells but also from the cast-off cytoplasmic material. When the sperm reaches maturity the acrosome disappears from view and the anterior edge of the head becomes well rounded.

In addition to the parts mentioned, there appears very frequently a dense spherical cytoplasmic mass represented in Figs. 67 to 75. As to the origin and fate of this body I am not entirely certain. It seems, however, that it is a portion of the centrosphere, for in many of the early stages it was seen in the immediate neighborhood of the small body which later forms the acrosome (Fig. 67). Further evidence for this assumption is the fact that the combined mass of these two bodies seems to be equal to the size of the sphere in the stage immediately preceding (Fig. 66). The consistency of this body, too, is similar to that of the sphere.

Figure 78 represents a mature spermatozoan of the pig which appears rather simple in structure and form. The entire nucleus of the spermatid has evidently developed into the head, which is oblong and flat (Figs. 70–78). The nuclear material breaks up into very fine particles which arrange themselves in a layer at the entire periphery of the head wall (Fig. 77).

With Heidenhain's iron-hæmatoxylin the head stains a sort of slate blue and is difficult to decolorize. A depression is present at the posterior extremity which is in contact with a small middle piece. The end-knob which is for some time attached to the nuclear wall within the small depression (Figs. 74, 75 and 76) finally breaks loose and passes to the posterior extremity of the middle piece, the greatest portion of which now remains very clear in appearance. This clear area is similar in appearance to the contents of the acrosome and one is led to believe that it is due to the presence of the substance which was seen in form of a clear sphere surrounding the centrosome after it emerged from the centrosphere and persisted about the two centrosomes, particularly around the inner one, after division (Figs. 66–69)

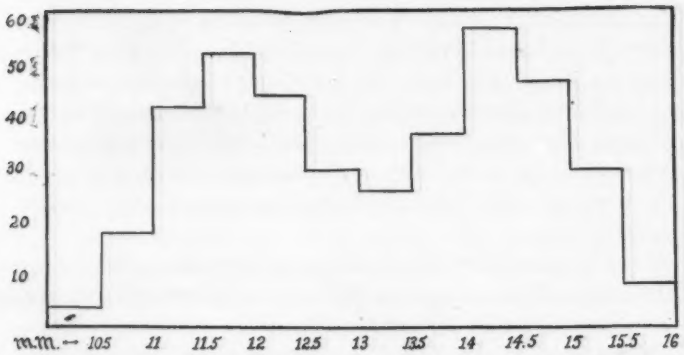

FIG. 1. Diagram showing the variation in size among four hundred mature pig spermatozoa. Figures at the left give the numbers of the individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

VARIATION IN SIZE OF MATURE SPERMATOZOA.

The spermatozoa of the pig vary considerably in size, and careful measurements revealed the fact that they may be arranged in two separate classes, one type being much larger than the other. Mature specimens, which were free in the lumen of the tubule and parallel to the objective, were selected at random, and outline sketches of four hundred heads enlarged (× 2,000) were made with the aid of a camera-lucida. The lengths of the sketches were then carefully measured and recorded in half millimeters. Figure 1 in the text shows the variation in size of the four hundred heads measured. It can be seen at a glance that two separate types of spermatozoa exist; the greatest number of the one kind measuring from 11.5 to 12 mm., and of the other type from 14 to 14.5 mm. The increased size of the latter is due presumably to the presence of the accessory chromosomes.

RELATION OF THE ACCESSORY CHROMOSOMES TO SEX IN THE PIG.

Many investigators working on the problem of sex determination are of the opinion that the sex of a zygote is determined at the time of fertilization, the spermatozoan in most cases carrying

the determining factor. This view has been recently substantiated by the careful investigations of McClung, Stevens, Wilson, Morgan, Payne and others, who found that in many invertebrates such as certain insects, myriopods, arachnids and nematodes, two different kinds of spermatozoa are produced. The spermatozoa differ from each other by one chromosome, or by a group of chromosomes, called the accessory chromosomes, or X-chromosomes.

In some forms it was found that the spermatozoa each contain an accessory chromosome, but that in half the sperms this chromosome was larger in its chromatin content than in the other. These are called the X and Y elements respectively. All the eggs produced (except in a few cases), on the other hand, contain the X-element.

It has been shown in some of the Tracheata that whenever an egg is fertilized by a sperm containing the X-chromosome it develops into a female, while an egg fertilized by a sperm without the X-element, or by one containing the Y-element, gives rise to a male individual.

In the vertebrates, Guyer was able to identify an X-element in such diverse forms as the guinea, rooster, pigeon, rat, and man, and other investigators have recently brought forth similar evidence. Patterson and Newman ('10) record its presence in the armadillo, Jordan ('11) in the opossum, Stevens ('11) in the guinea-pig, and King ('12) in *Necturus*. Up to the present investigation, however, a dimorphism in the number of the chromosomes in the male and female vertebrates has not been recorded. Guyer ('10) after speaking of the difference in the number of chromosomes in the somatic cells of the male and female Tracheata which possess the X-elements says:

"In the light of these facts we should expect the somatic cells of man to contain twenty-two and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above."

The behavior of the accessory chromosomes in the various stages of the spermatogenesis of the pig further substantiates the fact that two different kind of spermatozoa are produced in mammals. Since the dimorphic condition of the spermatozoa does exist, the question arose whether this dimorphism holds true in the chromosome number of the male and female somatic cells. With this in view I undertook an examination of sections of male and female embryological material supplied me by Professor B. M. Allen. A large number of these sections were studied and although mitotic stages were not very abundant in any one section, and the chromosomes were frequently bunched together rendering a count impossible, nevertheless, a number of counts of the chromosomes in the germinal and somatic cells of the two sexes were recorded. It was again found that the spermatogonial number of chromosomes is eighteen, and that the same number prevails in the somatic cells found in the mesonephros of the male embryos. Two of the chromosomes are usually somewhat larger (Fig. 59). Sections of the female material revealed a count of twenty chromosomes in the oögonia (Fig. 60), and the same number prevails in the somatic cells found in the mesonephros of the female embryos (Fig. 62). Four large chromosomes corresponding to the two accessories in the male can usually be detected in the oögonial cells. In the somatic cells of the female there are also four large chromosomes present corresponding to the two accessories in the male cells and the four accessories in the oögonia (Fig. 62).

In a few cases ten large chromosomes were found in the early metaphases of division in the somatic cells of the female (Fig. 61). Two of these are considerably larger and are interpreted as the result of the pairing of the four accessories. The smaller chromosomes, eight in number, judging by their size, are evidently due to the pairing of the sixteen other chromosomes. The position of these cells would not warrant the supposition that they might be wandering germ cells. Furthermore, a similar condition was not observed among the oögonial cells in spite of the fact that mitotic stages occur far more frequently in the sections of the ovaries than they do in the mesonephros.

In proportion to the large number of mitotic stages found in

these long-continued searches, accurate counts of chromosomes in the cells of the various tissues were comparatively few. However, all cases where a count was possible were recorded and although they varied somewhat, the above number of chromosomes, eighteen and twenty, attributed to the male and female cells respectively, were the prevailing numbers.

The foregoing facts are in accord with the expectations, and we have here in a vertebrate a condition verifying the results found in some of the lower forms. In view of these facts it is obvious that the eggs, carrying ten chromosomes, or half the somatic number, when fertilized by a spermatozoan containing ten chromosomes, give rise to an individual containing twenty chromosomes in its cells, or a female. Those fertilized by the other type of spermatozoan, which contains only eight chromosomes, give rise to individuals with eighteen chromosomes in their cells, which was found to be true in the male.

The results of the present investigation, therefore, add support to the chromosome theory of sex determination, since they show that in the vertebrates, as well as in some of the lower forms, there exists a dimorphism in the number of chromosomes in the somatic as well as the germinal cells of the two sexes. It is highly probable that conditions similar to those found in the pig, as regards sex determination, exist in man and in the other vertebrates which possess the accessory or X-chromosomes. The resemblance in the behavior of the pair of accessories in the pig and their behavior in man is very striking and suggests that in all probabilities there exists a dimorphism in the germinal and somatic cells of man and woman.

Summary.

1. The usual four types of cells, the spermatogonia, primary and secondary spermatocytes, and spermatids are discernible in the spermatogenesis of the pig.

2. Large interstitial cells containing numerous mitochondria exist in great abundance and comprise about one fourth of the entire mass of the testes.

3. Numerous Sertoli or nurse cells are present and great numbers of spermatozoa can be seen in all stages of development in the various tubules.

4. Two large nucleoli are present in the resting stages of the spermatogonia. These can be traced through the entire spermatogenesis of the pig and are apparently correlated with, or are the same thing as the two accessories. Smaller nucleoli, usually two, are also present.

5. Eighteen rod-shaped chromosomes differing somewhat in size occur in all of the spermatogonia where a definite count could be made. Two of these, undoubtedly the accessories, can usually be seen to one side of the main mass of chromosomes.

6. During the spermatogonial division the accessories divide and occasionally pass to the poles in advance of the other chromosomes.

7. The last spermatogonial division gives rise to cells which through a process of growth become the primary spermatocytes. Both nucleus and cytoplasm increase greatly in size. Synizesis followed by synapsis occurs.

8. An apparently continuous spireme is formed which later breaks up into U-shaped and variously curved chromosomes. A centrosphere containing the centrosome is present.

9. The two large round nucleoli which remain very conspicuous throughout the process of growth of the primary spermatocyte come together and elongate during the late prophase.

10. Ten chromosomes appear for division in the primary spermatocyte of which eight are evidently bivalent and two accessory. The accessories are usually considerably to one side of the other chromosome.

11. The two accessory chromosomes which are out of the main spindle pass undivided to one pole in advance of the other chromosomes.

12. The primary spermatocyte division is evidently the reduction division. It gives rise to two cells, one of which contains ten (eight autosomes plus the two accessories) and the other only eight chromosomes.

13. The chromosomes in these daughter cells are larger than the univalents of the spermatogonia and show some indications of bivalence.

14. The secondary spermatocytes formed by the last division ordinarily go into a resting stage. In half of these the two

nucleoli are conspicuous and lacking in the others. In a few cases, at least, there is no resting period in the secondary spermatocytes. A small centrosphere containing a relatively large centrosome is present.

15. During the metaphase of the secondary spermatocyte one half show four large bivalent chromosomes, and the remaining show the four large bivalents plus the two accessories or six chromosomes. A second pairing has apparently taken place but the division is simply equational as the four large chromosomes often manifest a quadrivalent character. The accessories remain unpaired.

16. The quadrivalent nature of the autosomes in the secondary spermatocytes becomes all the more certain after division, as the chromosomes passing to the poles are of the bivalent nature.

17. The type of secondary spermatocyte which received eight chromosomes after the first maturation division gives rise to two spermatids each containing four bivalent or eight univalent chromosomes. The other type which received ten chromosomes after the first maturation division gives rise to two spermatids, each containing four bivalent or eight univalent chromosomes and the two accessories, each accessory having divided here for the first time since the spermatogonial division.

18. The dimorphic nature of the spermatids which develop into spermatozoa is further evinced by the presence of the two nucleoli in approximately half the cells. The centrosome is again conspicuous.

19. The first noticeable change in the transformation of the spermatid is in the centrosome. It emerges from the small sphere and divides into an anterior rod-shaped and a posterior disc- or ring-shaped body.

20. Both of the centrosomes contribute to the development of the axial filament. A portion of the anterior one persists as the end-knob, while the posterior body is finally cast off.

21. A portion of the sphere migrates to the opposite side and gives rise to the acrosome which disappears when the spermatozoan is fully developed.

22. The nucleus of the spermatid passes to one side of the cell, elongates and flattens and forms the head of the spermatozoan.

23. The axial envelope which extends a little more than half the length of the tail is developed from the cytoplasm.

24. The half developed spermatozoa attach themselves to Sertoli cells where they continue to develop.

25. The cytoplasmic mass of the Sertoli cells decreases greatly in size as the spermatozoa continue to develop. Finally, when the spermatozoa are almost mature, a collapse of the nurse cells takes place when practically nothing but the nucleus and the cell wall remain.

26. An interesting event occurs in the final stages of the development of the spermatozoan when a large mass of cytoplasm together with the posterior centrosome is thrown off by the cell.

27. When the spermatozoa desert the Sertoli cells they do not pass directly into the lumen of the tubule but remain scattered with their heads embedded in the layer of the cast-off cytoplasm.

28. The cast-off cytoplasmic material is apparently used as food by the maturing spermatozoa and when practically all of the former disappears the fully developed spermatozoa become free in the lumen of the tubule.

29. Measurements of the mature spermatozoa reveal the fact that they are of two distinct sizes, a point no doubt correlated with the presence and absence of the accessory chromosomes.

30. The somatic cells of the male contain eighteen chromosomes, a number corresponding to that in the spermatogonia.

31. The somatic cells of the female yield a count of twenty chromosomes as do the oögonia.

32. The foregoing facts add considerably to the support of the chromosome theory of sex determination, since they prove that in the vertebrates, as is true of some invertebrates, there exists a dimorphism in the germinal and somatic cells of the male and female.

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EXPLANATION OF PLATES.

PLATE I.

(Fig. 1, ×200; Figs. 2 and 3, ×500; Figs. 4–12, ×1,200.)

1. Section of a pig testis showing the seminiferous tubules separated by wide areas of interstitial cells.

2. Section of a tubule showing bunches of developing spermatozoa attached to Sertoli or nurse cells.

3. Section of a tubule showing almost fully developed spermatozoa which have deserted the Sertoli cells and became scattered among the masses of cast-off cytoplasm.

4. Resting stage of a spermatogonial cell showing two large nucleoli.

5. Primary spermatocyte in the synizesis stage.

6. Resting stage of a primary spermatocyte showing the two large nucleoli.

7. Spireme stage of a primary spermatocyte showing the chromatin threads and the two persisting nucleoli.

8. Late prophase of division in a primary spermatocyte showing eight chromosomes in the main group, and the two accessories lying off to one side. The photograph does not reveal the full size of the accessories.

9 and 10. Side views of primary spermatocytes showing the cell ready for division with the accessories lying just above the level of the regular equatorial plate of chromosomes.

11. Side view of a primary spermatocyte division with the two accessory chromosomes passing to the pole, side by side, in advance of the other chromosomes.

12. Side view of a primary spermatocyte division showing the two accessories at the pole while the autosomes are still in the equatorial plate undivided.

13. Two metaphases of secondary spermatocytes showing four chromosomes in the equatorial plate. The figure at the top is a side view, and the one at the bottom a polar view.

14. The cell at the top is one type of secondary spermatocyte which contains four chromosomes in the metaphase stage and the cell below is a spermatid resulting from the division of a type of secondary spermatocyte which received eight autosomes plus the two accessories.

15. Resting stage of a spermatid showing two nucleoli.

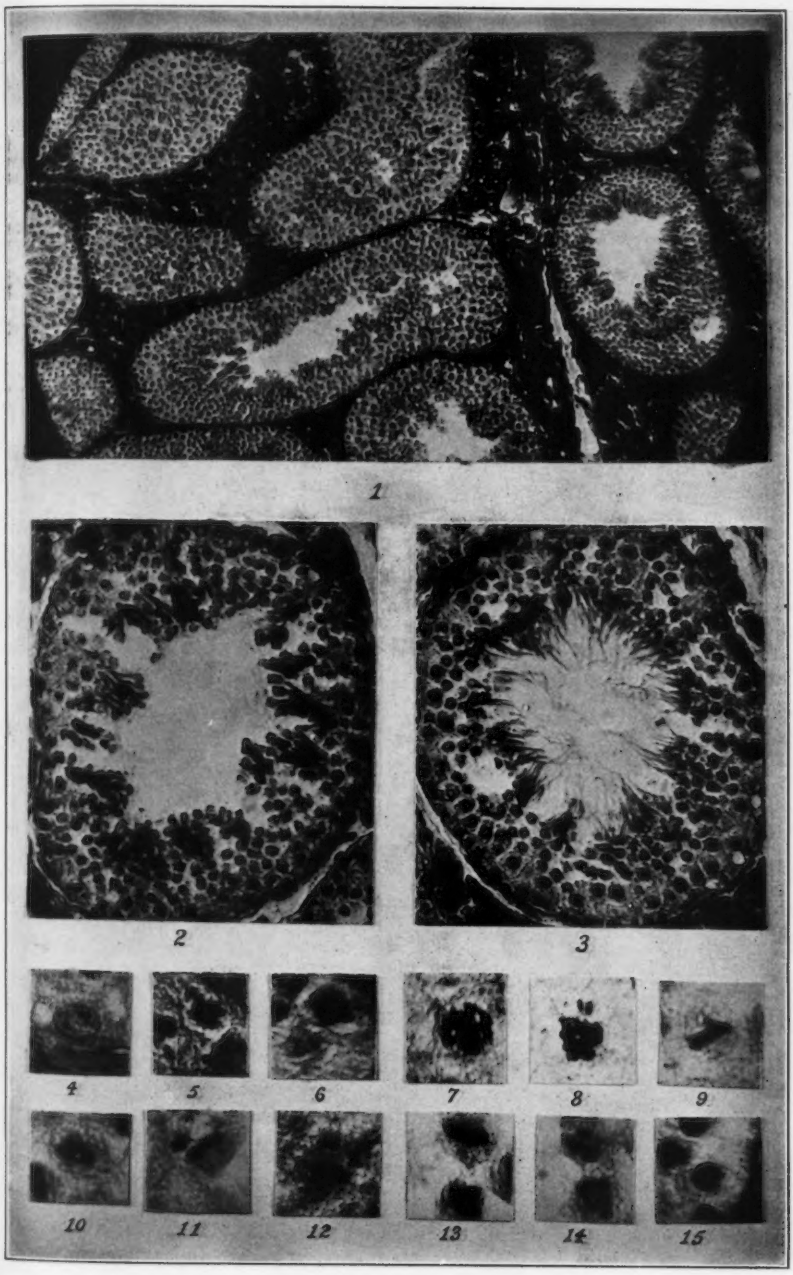

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PLATE II.

(All of the drawings on plates 2–6 were made with the aid of a camera lucida. ×2,330.)

16. Early spermatogonial cell showing two large nucleoli. The cell boundary is quite indistinct.

17. Resting stage of a full grown spermatogonial cell showing two large and two small nucleoli.

18. Late prophase of a spermatogonial division showing eighteen chromosomes. The two large oval chromosomes at the top are apparently the accessories.

19. Metaphase of division in a spermatogonium showing the two divided accessories at the right.

20. Early resting stage of a primary spermatocyte showing the centrosome, two large, and several small nucleoli.

21. Primary spermatocyte in synizesis.

22. Primary spermatocyte following synizesis and synapsis; the entangled mass of threads moved toward the center of the nucleus and the threads appear to be twice as thick as those in the synizesis stage.

23. Primary spermatocyte following the synaptic stage. The clear area in the nucleus disappears and the nuclear wall becomes well developed.

24. Spireme stage of a primary spermatocyte showing increase in size of both the nucleus and the cytoplasm and also the two large nucleoli.

25. Breaking up of the spireme into U and variously shaped chromosomes. The two nucleoli become oval in shape and can always be seen close together in this stage.

26, 27 and 28. Late prophases of primary spermatocytes showing ten chromosomes. The two oval accessories, which are smaller than the bivalent autosomes, are shown in characteristic positions.

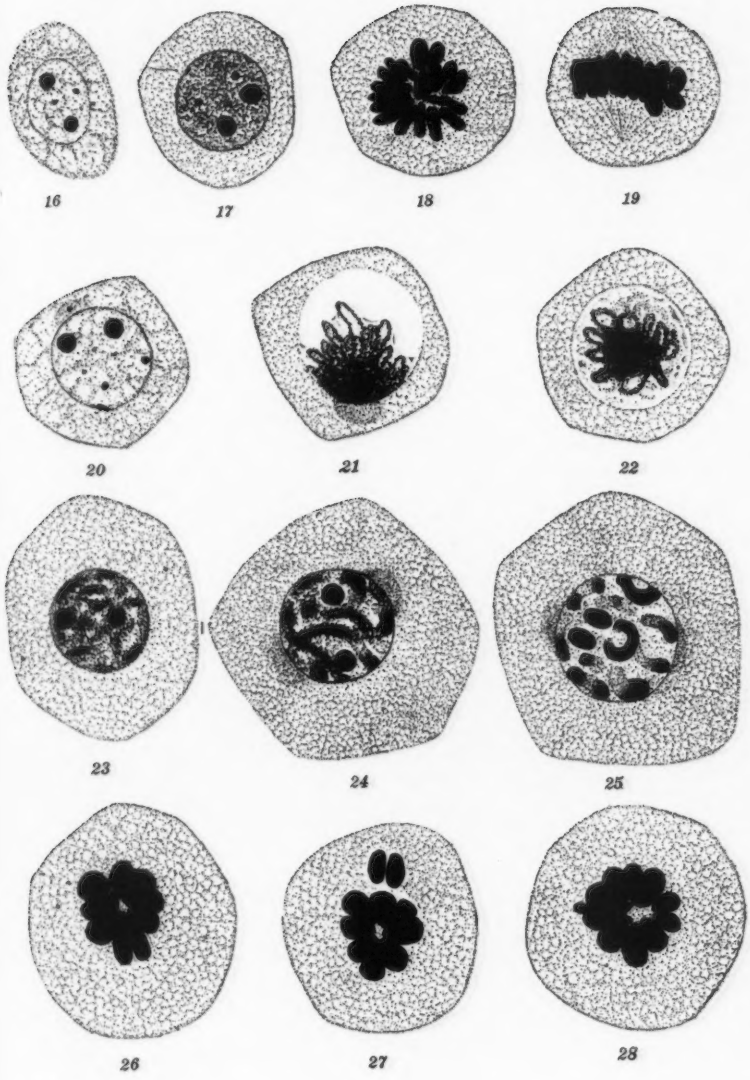

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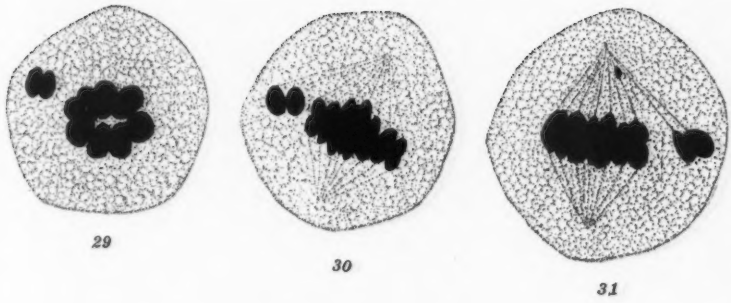
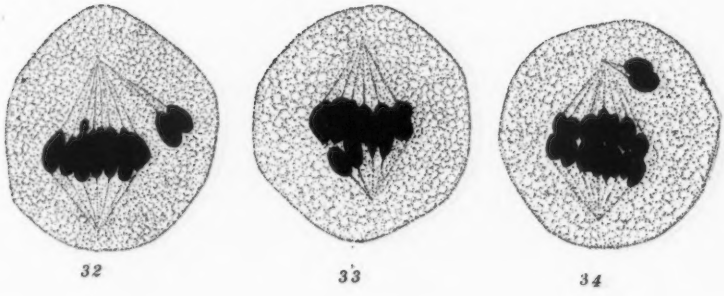
PLATE III.

29. Late prophase of a primary spermatocyte showing ten chromosomes, the two accessories lying a considerable distance away from the regular equatorial plate.

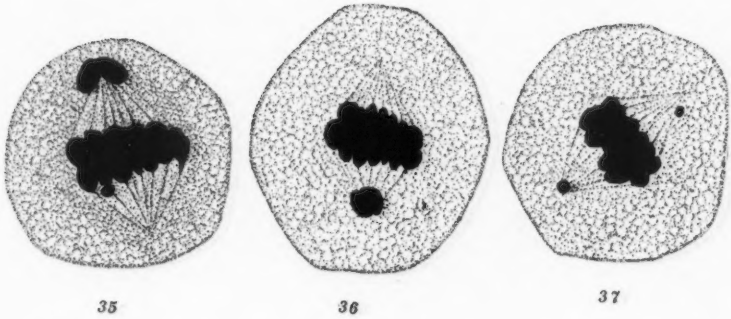
30–37. Metaphase of division in primary spermatocytes showing the two accessory chromosomes in characteristic positions passing to the poles. Figures 31 and 32 show also a small body passing to the same pole with the accessories, Figure 35 shows it passing to the opposite pole; and Fig. 37 shows two such bodies, one passing to each pole, which is a case of extremely rare occurrence.



29 30 31



32 33 34



35 36 37

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PLATE IV.

38. Late anaphase of division in a primary spermatocyte showing eight chromosomes at one pole and eight plus the two accessories at the other.

39. One end of a late anaphase of division in a primary spermatocyte showing eight chromosomes.

40. One end of a late anaphase of division in a primary spermatocyte showing ten chromosomes (eight plus the two accessories).

41. Resting stage of a secondary spermatocyte showing two nucleoli and a conspicuous centrosome.

42. Resting stage of a secondary spermatocyte without the large nucleoli.

43. Two contiguous secondary spermatocytes of which one shows four bivalent chromosomes in metaphase of division, and the other four bivalents plus the two accessory chromosomes in metaphase of division. These two cells are no doubt the products of a division of a primary spermatocyte in which eight chromosomes pass to one pole and eight plus the two accessories to the other.

44. Early metaphase of division in a secondary spermatocyte which received the two accessories, showing six chromosomes in all.

45. Early metaphase of division in a secondary spermatocyte which did not receive the accessories, showing only the four large bivalents.

46. Late prophase of division in a secondary spermatocyte which did not receive the accessories.

47. Late prophase of division in a secondary spermatocyte which received the accessories, showing a total of six chromosomes.

48. Late anaphase of division in a secondary spermatocyte which received eight chromosomes, showing four chromosomes at each pole.

49. A spermatid showing four bivalent chromosomes which is apparently one of the resulting cells of the division of a secondary spermatocyte which received eight chromosomes.

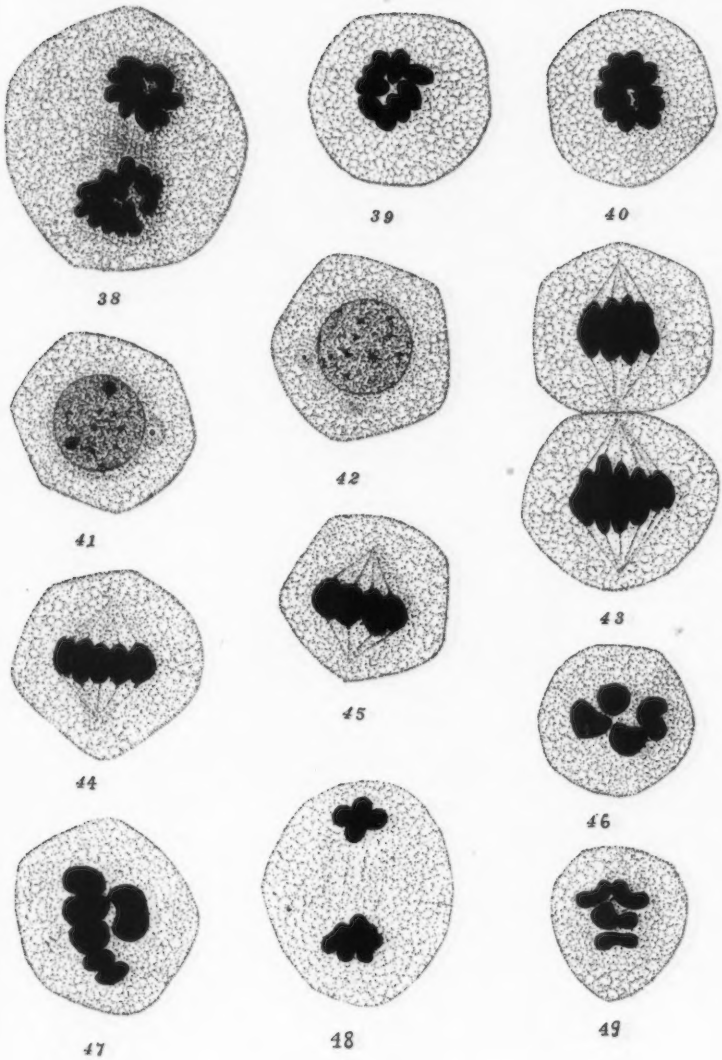

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PLATE V.

50 and 51. Late anaphase of division in a secondary spermatocyte which received the two accessories. Each of the latter divides in this stage. Figure 50 shows streaks of chromatin material uniting the two accessories. Usually no such streaks occur (Fig. 51).

52. One end of a late anaphase of a division in a secondary spermatocyte which received the two accessories. The latter are oval in shape and smaller than the bivalent autosomes.

53. A spermatid which received the accessories, showing a common arrangement of the chromosomes before they break up.

54. Division of a secondary spermatocyte which apparently received the accessories. The chromosomes are so massed as to render a count impossible and the two streaks which rarely occur are probably due to retarded division of the accessories.

55. An early spermatid in which the chromosomes have broken up, while the two nucleoli remain in full evidence and are apparently the same thing as the accessories.

56. An early spermatid, which received the accessories, showing a characteristic breaking up of the bivalent autosomes into univalents. Two of the autosomes had divided almost completely while the other two also show signs of division. The accessories are univalent in nature.

57. A spermatid, which did not receive the accessories, showing a common arrangement of the four bivalent chromosomes. Very frequently these autosomes divide before they become massed together, as is the case of the autosomes in the other type of spermatids represented in Fig. 56.

58. A spermatid showing the reconstruction of the nuclear wall while the four bivalent chromosomes are still in full evidence.

59. Late prophase of a somatic cell, found in the mesonephros of a male pig embryo, showing eighteen distinct rod-shaped and oval chromosomes two of which are somewhat larger and are apparently the two accessories.

60. Late prophase of an oögonial division showing twenty chromosomes, four of which are longer and evidently the accessories.

61 and 62. Somatic cells, found in the mesonephros of a female pig embryo. Figure 61 shows a very uncommon condition revealing ten large chromosomes which apparently resulted from the pairing of the twenty chromosomes. Two of these are considerably larger and are interpreted as the result of the pairing of the accessories. Figure 62 shows the late prophase of division in a type of somatic cell occurring most frequently, and reveals a count of twenty chromosomes four of which are considerably larger than the others.

63. A full-grown Sertoli or nurse cell.

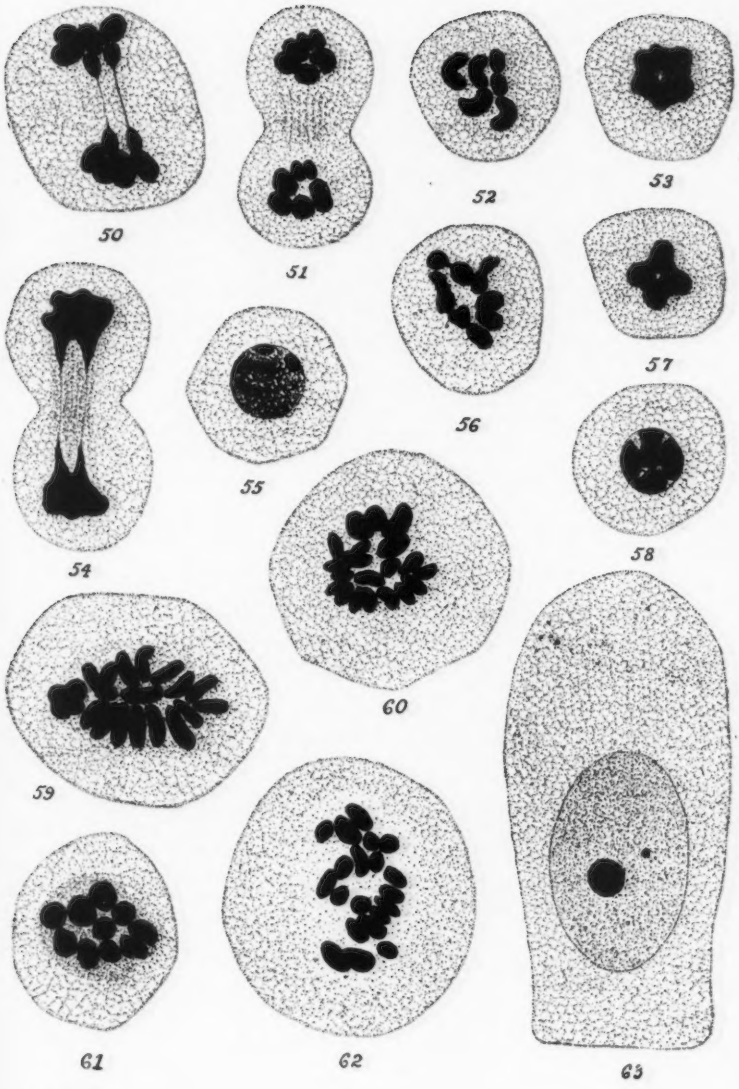

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PLATE VI.

64 and 65. Two contiguous spermatids, one without chromatin nucleoli, the other with two. The spermatids in general are about equally divided into these two classes. The centrosome is very conspicuous in this stage.

66. A spermatid showing the large centrosome, surrounded by a clear area, which had emerged from the sphere a short distance away.

67. A spermatid showing the centrosome in contact with the nuclear wall, and the migration of a portion of the sphere toward the opposite pole.

68. A spermatid showing the division of the centrosome, the position of the sphere as the acrosome, and the migration of the nucleus toward one side of the cell.

69. A transforming spermatid showing the separation of the centrosomes and the beginning of the axial filament. Also a dense spherical cytoplasmic body which is likewise present in some of the following stages.

70. A transforming cell showing a characteristic ring-like structure and position of the posterior centrosome with the axial filament extending through it.

71. A later stage showing the sloughing off of the posterior ring-shaped centrosome from the axial filament.

72. A somewhat later stage showing the posterior centrosome completely separated from the axial filament.

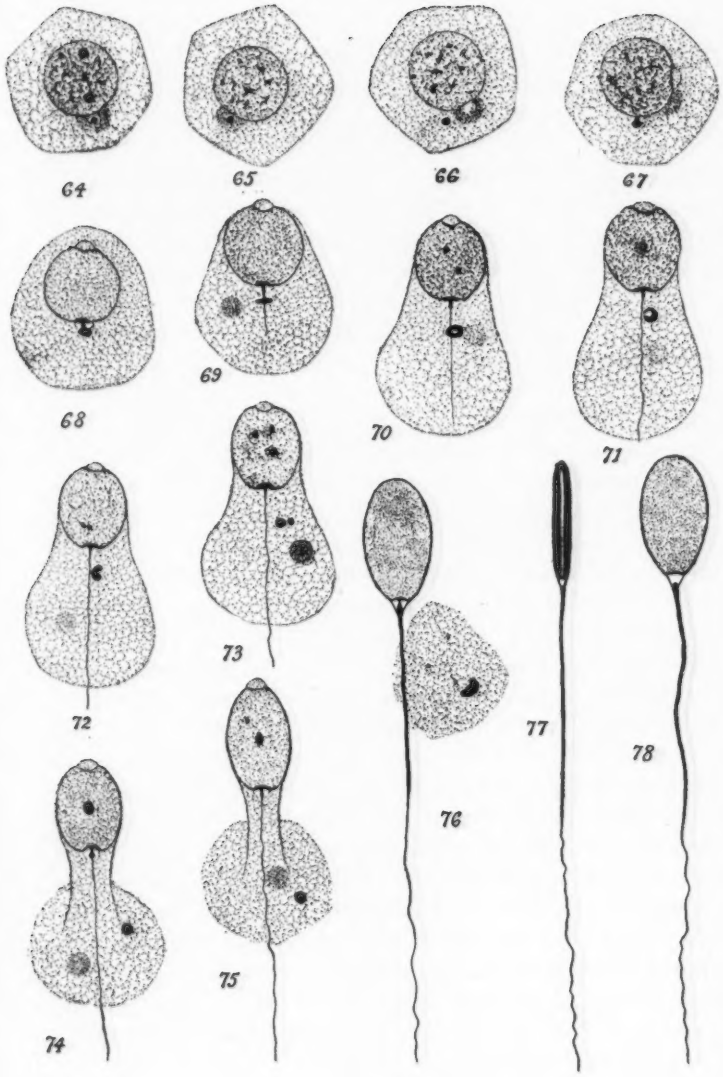
73. A cell showing the cast-off centrosome divided in two.

74 and 75. Still later stages of the developing spermatozoan showing the characteristic position of the posterior centrosome, and the formation of the axial envelope.

76. A spermatozoan almost fully developed showing the mass of sloughed off cytoplasm including the centrosome.

77. Side view of a mature spermatozoan showing the arrangement of the chromatin at the periphery of the head wall.

78. A mature spermatozoan.



J. G. WOODSDALE

A NEW EYE COLOR MUTATION IN DROSOPHILA AND ITS MODE OF INHERITANCE.

SHELLEY R. SAFIR.

A cross between a long-winged, vermilion-eyed female and a miniature-winged, red-eyed male yielded in the first generation red-eyed females and vermilion-eyed males, both long-winged. There appeared, however, in addition 15 long-winged males with a new eye color. This color is of the same general tone as the vermilion pink eye but is paler and somewhat creamier. A lighter area may also be seen encircling the darker area of the center.

The origin of this new eye color was accounted for as follows: Since vermilion is sex-linked, all the sons must be vermilion whatever else they may be. Only males of the new eye color appeared, wherefore it was inferred that the something else added was also sex-linked. There were too many of them (15) to be explained each as a separate mutation, and it seems clear that the mutation itself occurred back in the stock from which the mothers were taken. If one or more of these P_1 vermilion females were heterozygous for the new sex-linked factor we should expect in F_1 several of the double recessive males of the composition cherry vermilion. (It was decided to call the new eye color cherry vermilion.)

If then this eye color represents a double recessive cherry vermilion, when mated to red eye (the normal eye color) there should result in F_1 only red-eyed offspring. Then in F_2, if both of these factors are sex-linked, there should appear four classes of males, viz., the double recessive cherry vermilion, and cherry and vermilion as the result of splitting cherry vermilion into its components, and also red eye color which is the double dominant. All the females should be red. That these results were actually realized can be seen below.

red ♀ × cherry verm. ♂ = { red ♀ 762 / red ♂ 758 } { red ♀ ..... 345 / red ♂ ..... 114 / cherry ♂ ..... 41 / verm. ♂ ..... 54 / cherry verm. ♂ .... 120 }

The four classes of males taken together number 329, which approximates to the 345 red females. The results may be accounted for in the following way, using the new system of symbols proposed by Morgan (1912).

P1 red ♀ ..... XCV — XCV
cherry verm. ♂ ... Xcv — ——

F1 FEMALE. XCV, Xcv, red ♀ F1 MALE. XCV, ——, red ♂

Gametes of F1 ♀ XcV — XCV — Xcv — XCv
Gametes of F1 ♂ XCV — ——

F2 FEMALES.		F2 MALES.	
XcV / XCV	red ♀	XcV / ——	cherry ♂
XCV / XCV	red ♀	XCV / ——	red ♂
Xcv / XCV	red ♀	Xcv / ——	cherry verm. ♂
XCv / XCV	red ♀	XCv / ——	verm. ♂

It will be noted that the red-eyed females belong to four classes pure red, reds heterozygous for vermilion, reds heterozygous for cherry, and reds heterozygous for vermilion and cherry. The red and cherry vermilion male classes are each about twice as numerous as the cherry and the vermilion classes respectively. This disturbance in the ratio is due to linkage between cherry and vermilion.

CHERRY VERMILION BY VERMILION.

When vermilion-eyed females were mated to cherry vermilion-eyed males all the offspring, 483 in number, were vermilion. These when inbred produced vermilion females, vermilion males and cherry vermilion males.

verm. ♀ by cherry verm. ♂ = { verm. ♀ 252, verm. ♂ 231 } > { verm. ♀ ..... 374, verm. ♂ ..... 158, cherry verm. ♂ ... 158 }

The results bear out the view that the new eye color represented the double recessive; vermilion plus some other sex-linked factor, viz., cherry, as the analysis shows:

P1 verm. ♀ ..... XCv — XCv
cherry verm. ♂ ..... Xcv — ——

F_1 Female.	F_1 Male.
XCv / Xcv	XCv / ——
verm. ♀	verm. ♂

F_2 Females.		F_2 Males.	
XCv / XCv	vermilion ♀	XCv / ——	vermilion ♂
Xcv / XCv	vermilion ♀	Xcv / ——	cherry verm. ♂

The expectation is as many vermilion as cherry vermilion males and the count shows this is exactly realized.

Cherry Vermilion by Pink.

When pink-eyed females were mated to cherry vermilion males all the offspring, 381 in number, were red. These, when inbred, should produce red, cherry, vermilion, pink, cherry vermilion, cherry pink, vermilion pink and cherry vermilion pink males. Because of difficulty of separation the male classes vermilion pink, cherry pink, cherry vermilion, and cherry vermilion pink were put into one general class. The females were of two classes only, viz., red and pink. The following analysis gives the expectation:

pink ♀ × cherry verm. ♂ = { red ♀ / red ♂ } >

red ♀	330
pink ♀	79
red ♂	87
cherry ♂	40
verm. ♂	48
pink ♂	29
cherry verm. ♂	126
cherry pink ♂	
verm. pink ♂	
cherry verm. pink ♂	

The eight classes of males taken together number 430 as compared with 409 red and pink females. The results may be accounted for in the following way:

P_1 pink ♀ ..... XCVp — XCVp
cherry verm. ♂ ..... XcvP — ——P

F_1 Female.	F_1 Male.
XCVp / XcvP	XCVp / ——P
red ♀	red ♂

Gametes of F_1 ♀.

XcVp — XCVp — XcvP — XCvP
XcVP — XCVP — Xcvp — XCvp

Gametes of F_1 ♂.

XCVP — XCVp — ——P — ——p

F_2 Females.	F_2 Males.
XcVp - XCVP....red ♀	XcVp - ——P....cherry ♂
XcVP - XCVP....red ♀	XcVP - ——P....cherry ♂
XCVp - XCVP....red ♀	XCVp - ——P....red ♂
XCVP - XCVP....red ♀	XCVP - ——P....red ♂
XcvP - XCVP....red ♀	XcvP - ——P....cherry verm. ♂
Xcvp - XCVP....red ♀	Xcvp - ——P....cherry verm. ♂
XCvP - XCVP....red ♀	XCvP - ——P....verm. ♂
XCvp - XCVP....red ♀	XCvp - ——P....verm. ♂
XcVp - XCVp....pink ♀	XcVp - ——p....cherry pink ♂
XcVP - XCVp....red ♀	XcVP - ——p....cherry ♂
XCVp - XCVp....pink ♀	XCVp - ——p....pink ♂
XCVP - XCVp....red ♀	XCVP - ——p....red ♂
XcvP - XCVp....red ♀	XcvP - ——p...cherry verm. ♂
Xcvp - XCVp....pink ♀	Xcvp - ——p...cherry verm. pink ♂
XCvP - XCVp....red ♀	XCvP - ——p....verm. ♂
XCvp - XCVp....pink ♀	XCvp - ——p....verm. pink ♂

It will be noted that the expectation is three red females to one pink female, but the actual count, because of disturbances due to viability, is four red females to one pink female. The male count does not agree with simple expectation in two instances. Both the vermilion and cherry classes which should be as numerous as the red (and the cherry vermilion respectively) are much less numerous than expectation. This, however, is due to coupling between cherry and vermilion.

Cherry Vermilion by Vermilion Pink.

When vermilion pink females were mated to cherry vermilion males all the offspring, 635 in number, were vermilion. These when inbred produced vermilion females, vermilion pink females, vermilion males, cherry vermilion males, vermilion pink males and cherry vermilion pink males. Because of difficulty of separation I have included the last two classes in a general class.

verm. pink ♀ × cherry verm. ♂ = { verm. ♀, verm. ♂ } > { verm. ♀ 509; verm. pink ♀ 208; verm. ♂ 223; verm. pink ♂ 89; cherry verm. ♂ + verm. pink cherry ♂ 319 }

The four classes of males taken together number 631; the vermilion and the vermilion pink (orange) females number 717.

P_1	verm. pink ♀	XCvp	—	XCvp
	cherry verm. ♂	XcvP	—	——P

	F_1 Female		F_1 Male.	
	XCvp XcvP verm. ♀		XCvp ——P verm. ♂	
Gametes of F_1 ♀	XCvp	XcvP	XCvP	Xcvp
Gametes of F_1 ♂	XCvp	XCvP	——P	——p

F_2 Females.		F_2 Males.	
XCvp XCvp	verm. pink ♀	XCvp ——P	vermilion ♂
XcvP XCvp	vermilion ♀	XcvP ——P	cherry verm. ♂
XCvP XCvp	vermilion ♀	XCvP ——P	vermilion ♂
Xcvp XCvp	verm. pink ♀	Xcvp ——P	cherry verm. ♂
XCvp XCvP	vermilion ♀	XCvp ——p	verm. pink ♂
XcvP XCvP	vermillon ♀	XcvP ——p	cherry verm. ♂
XCvP XCvP	vermilion ♀	XCvP ——p	vermilion ♂
Xcvp XCvP	vermilion ♀	Xcvp ——p	cherry verm. pink ♂

The count shows that the vermilion pink females in spite of their low viability ran above expectation. The males fall into four classes, two of which can be easily separated out. The cherry vermilion pinks, however, are difficult to distinguish from the cherry vermilions and are therefore included in that class. Together they should be four times as numerous as the vermilion pink class, and the vermilion class ought to be three times as numerous as the latter, which is actually the case.

Cherry Vermilion by White.

When white-eyed females were mated to cherry vermilion-eyed males all the male offspring the first generation, 363 in number, were white. The females, 392 in number, were cherry. These when inbred produced in the second generation, white females, cherry females, white males, cherry males and cherry vermilion males.

white ♀ by cherry verm. ♂ = { white ♂ 363 / cherry ♀ 392 } {
white..... ♀ 347
cherry..... ♀ 334
white..... ♂ 321
cherry..... ♂ 112
cherry verm..... ♂ 222
}

P_1	white ..... ♀	XcVw — XcVw
	cherry verm. ..... ♂	XcvW — ——
	F_1 Female.	F_1 Male.
	XcVw	XcVw
	XcvW	——
	cherry ♀	white ♂
Gametes of F_1 ♀	XcVw — XcvW — XcVW — Xcvw	
Gametes of F_1 ♂		XcVw — ——
	F_2 Females.	F_2 Males.
	XcVw / XcVw white ♀	XcVw / —— white ♂
	XcvW / XcVw cherry ♀	XcvW / —— cherry verm. ♂
	XcVW / XcVw cherry ♀	XcVW / —— cherry ♂
	Xcvw / XcVw white ♀	Xcvw / —— white ♂

The expectation is an equal number of white females and cherry females. The actual count was 347 white females and 334 cherry females. Of the three classes of males the expectation is as many white males as cherry and cherry vermilion put together which is the case as the figures show: 321 white males and 334 cherry and cherry vermilion males. The cherry and cherry vermilion males should be in equal numbers. But this is not the case in the actual count as the cherry vermilion males are twice as numerous as the cherry males: 222 of the former to 112 of the latter. This effect is due to linkage.

Cherry Vermilion by Eosin.

When eosin-eyed females were mated to cherry vermilion-eyed males all the male offspring in the first generation were eosin and the females were cherry. These when inbred produced in the second generation, eosin females, cherry females, eosin males, cherry males, cherry vermilion males, and eosin vermilion males.

Because of difficulty of separation, I have placed the cherry and the eosin females in one general class. For the same reason I have placed the eosin and the cherry vermilion males in one general class.

eosin ♀ by cherry verm. ♂ = { eosin ♂ 119 / cherry ♀ 154 } { eosin ♀ ..... / cherry ♀ ..... } 110 ; { eosin ♂ ..... / cherry ♂ .....20 / cherry verm. ♂ ..... / eosin verm. ♂ .....18 } 76

The results may be accounted for in the following way:

P eosin..... ♀ XcVe — XcVe
cherry verm..... ♂ XcvE — ——

F1 FEMALE. F1 MALE.
XcVe XcVe
XcvE ——
cherry ♀ eosin ♂

GAMETES OF F1 ♀ XcVe — XcvE — Xcve — XcVE
GAMETES OF F1 ♂ XcVe — ——

F2 FEMALES.		F2 MALES.	
XcVe / XcVe	eosin ♀	XcVe / ——	eosin ♂
XcvE / XcVe	cherry ♀	XcvE / ——	cherry verm. ♂
Xcve / XcVe	eosin ♀	Xcve / ——	eosin verm. ♂
XcVE / XcVe	cherry ♀	XcVE / ——	cherry ♂

In concluding I wish to express thanks to Dr. Morgan for furnishing me with material with which to carry on these crosses and for his helpful suggestions during the progress of the work. I have also been greatly helped by discussion of the theoretical side of the matter with Messrs. A. H. Sturtevant, H. J. Muller and C. B. Bridges. Mr. Bridges' suggestions and his final reading and criticism of the manuscript were directly responsible for the appearance of the paper in its present form. I must not forget to express my appreciation of the assistance of my friend Benjamin Schwartz.

PARTHENOGENETIC CLEAVAGE OF THE ARMADILLO OVUM.

H. H. NEWMAN.

Introduction.

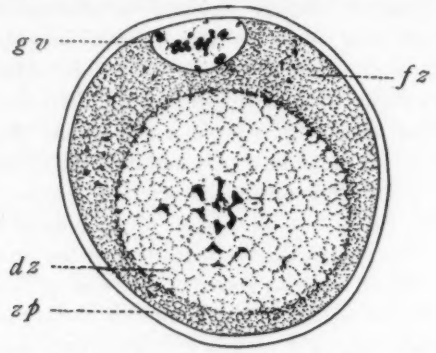
In the year 1909 I began a general study of the ovary of the nine-banded armadillo in search of some explanation of the phenomenon of polyembryony. Among the first of the peculiar features to catch my attention were what appeared to be cleavage stages in certain types of ovarian ova. It was thought that this condition might have some bearing on polyembryony but further study and a review of the literature on kindred topics convinced me that these changes were in no sense to be interpreted as stages in normal development. They must, on the contrary, be interpreted as more or less abnormal and abortive attempts, on the part of ovocytes that have reached maturity but have been denied the normal culmination of their destiny in ovulation and fertilization, to develop embryos in spite of insuperable difficulties. I should hesitate to contribute another chapter to the already voluminous literature on the supposed parthenogenesis of mammalian ova during follicular atresia had I not at my command evidence of a very crucial character, which seems to me to demonstrate beyond controversy a certain amount of real parthenogenesis in a mammal.

The ovaries studied are the same as those that formed the basis of a recent paper on maturation and fertilization in this species (Newman, 1912). Those ovaries, fixed in freshly made Zenker's fluid and stained by Bensley's copper chrome hæmatoxylin process, give the clearest pictures.

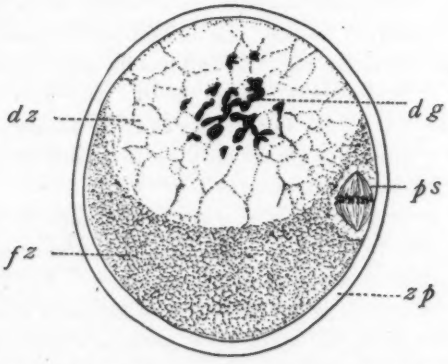
Résumé of the Events of Maturation Leading up to Parthenogenetic Cleavage.

In order that the changes preceding cleavage may be understood it will be necessary to review the facts dealing with the maturation process which were described in a recent paper (Newman, 1912). The most striking peculiarities of this process

have to do with the reorganization of the cytoplasmic materials of the ovocyte. In this respect, and probably in many others, the armadillo bears a marked resemblance to the marsupials, especially *Dasyurus*, the Australian native cat, whose early develop-



TEXT-FIG. 1. A full-grown ovocyte, showing cytoplasmic organization, etc. Deutoplasmic zone (*dz*), formative zone (*fz*), germinal vesicle (*gv*), zona pelucida (*zp*) (× 410).



TEXT-FIG. 2. A maturing ovocyte, showing the new reversed polarity. The ovocyte is placed with the animal pole upwards. The deutoplasmic zone (*dz*) occupies the animal pole, the formative zone (*fz*) occupies the vegetative pole. The polar spindle (*ps*) lies in a tangential position at the equator of the ovocyte. Deutoplasmic granules (*dg*) lie in the center of the deutoplasmic mass. The zona pelucida (*zp*) is a dense envelope, without radiations.

ment is so ably described by Hill (1910). So close is this resemblance that any description given for one species would apply in almost every detail to the other.

The full grown ovocyte of the armadillo has the structure shown in text-figure 1. The cytoplasm shows two clearly defined regions, a peripheral zone of deeply staining homogeneous protoplasm, the *formative zone* (*f.z.*), and a central lightly staining, spherical mass of coarsely reticular or alveolar material, the deutoplasmic zone (*d.z.*), in which occur irregular masses of coarse and deeply staining granules (*d.g.*), the supposed equivalent of the yolk or deutoplasmic granules of the ova of marsupials. The germinal vesicle (*g.v.*) is flattened against the dense zona pelucida (*z.p.*) at the animal pole and in it the chromatin is seen as tetrads or in other forms characteristic of the prophases of maturation. Such an ovum has the appearance of being centrolecithal. Coincident with the formation of the first maturation spindle a radical rearrangement of the two cytoplasmic zones occurs. The deutoplasmic sphere pushes its way to the surface at the animal pole and crowds the formative material to the vegetative pole where it assumes the form of a cap, thick at the pole and thinning out to a feather edge at the equator (see text-figure 2). The polar spindle, in a stable metaphase (*p.s.*), occupies a position as near the animal pole as possible without leaving the formative material and has its axis tangential to the periphery of the ovocyte though parallel with that of the main axis of the cell. This spindle is evidently insulated from the surrounding formative protoplasm by a capsule of non-staining material, and has no astral rays.

A very large number of ovocytes were found in exactly this condition and it seems certain that the progressive changes normally come to a standstill at this point. The next step in normal development is ovulation, the stimulus of which initiates the completion of the maturation process. Such resting ovocytes occur in large follicles whose granulosa cells are intact and whose follicular fluid is homogeneous, abundant, and stains deeply with hæmatoxylin and allied stains. In any normal ovary during the period of œstus there are many ovocytes of this sort, all in practically the same condition and equally ready for ovulation. Probably such mechanical factors as size of follicle or nearness to the periphery determine which follicle shall rupture first. Fertilization of this one egg seals the fate of all the remaining

ovocytes of the same generation in that they can never go through the process of ovulation and therefore cannot develop normally. A very small percentage of these ovocytes continue the maturation process to the extent of completing the first maturation division; a still smaller proportion (only three out of hundreds of cases examined) complete the second maturation; while the remainder, over 90 per cent., are struck by the processes of follicular atresia and either go into early cytolysis or enter upon a period of parthenogenesis. It is with the latter contingency that the present paper deals.

Abstriction of the Deutoplasmic Material and Its Subsequent Fate.

The condition of equilibrium, just described and illustrated in text-figure 2, is normally disturbed only by ovulation, but, in the case of ovocytes whose normal history has been cut short by the fertilization of an ovum and the formation of a corpus luteum, the equilibrium may also be upset by the marked changes in the chemical environment incident to follicular atresia. The follicle in which atresia has set in rapidly decreases in size through the loss of follicular fluid, but, even before the amount of fluid has materially diminished, its chemical composition is clearly altered, for it no longer stains deeply with the ordinary cytological stains but remains pale gray under hæmatoxylin treatment. Just what the nature of the change is I am unable to determine, but there can be no question as to its radical character. As atresia continues, the lumen of the follicle gradually disappears, partly through the loss of fluid and partly through the ingress of cells from the disintegrating granulosa layer of the follicle. With this radical alteration of chemical environment the ovocytes show marked changes, whose significance it is our problem to determine.

The first act of the ovocyte entering upon parthenogenetic development is the abstriction of the deutoplasmic mass from the formative protoplasm. That this is a practically universal phenomenon can scarcely be doubted for there are in the present material literally hundreds of instances of it, and one can with confidence look for some stage of the process in every follicle

that has reached the mid-period of atresia. Abstriction of the deutoplasm is essentially an act of rejuvenation on the part of a dying cell, in that the still living protoplasm is freed from the burden of the inert by-products of metabolism. The exact mechanics involved in this purification process can scarcely be determined from the fixed material, but appearances seem to warrant the conjecture that the very fluid deutoplasmic material escapes from the cell membrane by the rupture of the latter and that, with the subsequent closure of the ruptured membrane, the deutoplasm becomes strictly extra-cellular and without effect, except in a secondary mechanical way, upon the living protoplasm of the germ cell. Freed from its burden the renewed egg-cell rounds up into an approximately spherical form and apparently floats in the deutoplasmic fluid. It is of considerable interest to note that a similar abstriction of deutoplasmic material occurs as the initial step of normal development in *Dasyurus*. Hill shows that this discarded material plays no further part in development but merely occupies any available space within the persistent zona pelucida that is not occupied by the blastomeres. Finally it comes to lie in the cleavage cavity of the blastula and is probably gradually absorbed. In view of the striking similarity in the early history of the deutoplasm in the two species it is difficult to avoid the conclusion that the process, as described for these ovarian ova of the armadillo during atresia, is a close approximation of the first step in normal development, about which we know practically nothing at present.

The deutoplasmic fluid, laden with its masses of deutoplasmic granules, usually gives the appearance of a multicellular body, but I am inclined to consider this condition as largely an artefact. It is not improbable that the strong fixing fluids used coagulate this somewhat viscous material in the form of many small rounded masses. In a few cases, as in Figs. 2 and 4, the deutoplasm is only slightly broken, but in the majority of instances we find advanced fragmentation, as in Figs. 1, 3, 5, etc. Whether the deutoplasm occurs in one or many fragments the deutoplasmic granules occupy a central position in the various pieces giving each fragment the illusory appearance of a nucleated cell. These are not however to be interpreted as in any sense true

cells. At best they may be designated as *cytoids*. Other writers who have studied similar conditions in mammals have referred to the existence of multicellular conditions in which some cells possess nuclei and others do not. I suspect that, were the truth known, these fragments without nuclei would turn out to be of deutoplasmic origin. Very commonly these cytoids in our material form a pseudo-epithelial layer surrounding the true cells, as in Figs. 3 and 5, or less distinctly in Fig. 1. In other cases they are distributed at random among the cellular products of cleavage (Figs. 7–11) and serve only to give a false appearance of cellular multiplicity and to confuse and obscure the pictures of cleavage.

The Reëstablishment of Normal Interrelations Between the Nucleus and the Cytoplasm and the Resultant Formation of Cleavage Spindles.

The egg, much reduced in size but with a renovated cytoplasm, is now the seat of a renewal of nucleo-cytoplasmic exchanges, as evidenced by the appearance of an extensive system of astral rays at the two poles of the spindle. It will be recalled that the nucleus during the period prior to ovulation was a naked spindle, enclosed in an insulating capsule and entirely devoid of astral radiations. Such a spindle is evidently an isolated system and lies inert in the cytoplasm. The renovated cytoplasm of the egg from which the deutoplasm has been extruded now bears a different chemical relation to the nucleus and the change is shown in the disappearance of the capsule about the nucleus and in the appearance of typical astral radiations. That a renewal of metabolic relations between nucleus and cytoplasm is one of the most essential facts of normal fertilization has been recently maintained by Lillie (1911) on the basis of his studies of fertilization in *Nereis*, and there is reason to believe that this is the physiological explanation of parthenogenesis wherever the latter is found, whether normal or experimental. In this case it is my conviction that the sudden change in the chemical character of the cytoplasm restores the basis of life and growth to the egg and that cleavage is the natural consequence. In such cases as that shown in Fig. 1 the nucleus has returned to a

resting state and has every appearance of undergoing healthy changes which should culminate in cell division by mitosis. It is impossible to state positively that such a nucleus has regressed from the spindle condition shown in text-figure 2, but that such is the case seems at present to be a reasonable conjecture. In eggs of this sort there is a characteristic zone of activity in the cytoplasm at a short distance from the nuclear membrane which may indicate active interchanges between nucleus and cytoplasm. The chromatin is in the form of a series of beaded threads resembling a spireme and therefore may be interpreted as in a very early prophase of the first cleavage. Soon the chromosomes begin to condense and the typical appearances of later prophases pass over into unmistakable mitotic figures like that shown in Fig. 4, which can be interpreted in no other way than as cleavage spindles. The so-called cleavage mitoses of other writers on this subject have been interpreted by opponents as belated maturation spindles which have been displaced and distorted by the abnormal conditions of atresia; but such an interpretation of the figures here described would seem very far-fetched in view of the fact that these spindles are so radically different from the naked, insulated spindles of maturation. I have never seen a vestige of astral radiations in a polar spindle, while the radiations in a number of such cases as that shown in Fig. 4 are as obvious and as extensive as those which I have observed in the cleaving eggs of annelids and other favorable material, and far clearer than similar appearances in fish eggs or other less favorable material. The spindles are frequently abnormal in form, being not uncommonly tri- or multipolar; and sometimes the poles are ill defined, as in Fig. 4, but the radiations are always very distinct. When the figure is multipolar the chromatin distribution is very irregular and nests of nuclei (like those shown in Fig. 12) are produced without any division of the cytoplasm. It is very common to find such multinucleate but unicellular eggs and I am inclined to attribute their existence to such irregular mitoses as that just mentioned. Certain considerations lead me to venture a conjecture as to the mode of origin of these multipolar spindles. It is not uncommon to find in ovocytes of the type shown in text-figure 2 various steps

in the anaphases of the first maturation division, which might readily result in the formation of two nuclei without the extrusion of a polar-body. Conditions like those shown in Figs. 2 and 3 are not nearly so abundant as those like Fig. 1, but they are about as common as are multipolar spindles and might readily result in the latter type of abnormality. Eggs with such paired nuclei have the general appearance of fertilization stages but cannot be interpreted as such, unless the reunion of the polar nucleus with its sister, the nucleus of the secondary ovocyte, could be considered a sort of fertilization process. It is not difficult, however, to see how double nuclei of this sort could form just such multipolar spindles as that shown in Figs. 13 and 14. It will be readily noted from the figures that these abnormal spindles have a very much larger number of chromosomes than sixteen, the number seen in all clear maturation figures. This in itself militates against the interpretation of these phenomena as maturation processes, and is in accord with the conjecture that the figures may be the result of the coöperation of two nuclei derived from a maturation division.

The bipolar spindles, such as that shown in Fig. 4 (see also Figs. 15 and 16), also contain more than the haploid number of chromosomes and cannot on that account be maturation figures.

Early Cleavage Stages Resulting in the Formation of a Few Blastomeres.

In the course of these studies several very pretty two-cell stages have been encountered of which those shown in Figs. 5 and 6 are typical. The stage shown in Fig. 5 is a perfect two-cell stage in which both nuclei are in the resting phase. There are no other nucleated bodies within the zona pelucida, but a pseudo-epithelium of deutoplasmic cytoids forms a capsule about the two blastomeres. A clear case of a two-cell stage in which the second cleavage is well under way is shown in Fig. 6. The cleavage spindles are cut transversely in both cells, one of which shows an early and the other a later anaphase.

After the second cleavage the subsequent divisions are less normal and the picture of blastomeric regularity is confused. The spindles lose distinctness, as a rule, and the chromatin

begins to break down, giving appearances like those shown in Figs. 7 and 8, which are two sections through the same egg. Another example of the same condition is shown in Figs. 9 and 10, also taken from one egg. In Fig. 7 can be seen one cell with a fragmenting nucleus, a degenerating nucleus in another cell, and an incomplete mitotic figure with only a few small chromosomes. In Fig. 8 occur three cells all of which show abnormal, though unmistakable, mitotic figures. Such an egg might be considered as a six-cell stage, with a prospect of reaching a ten-cell condition. There is every evidence, however, of approaching death and disintegration in such cases and one would not be inclined to look for much further developmental progress in such unpromising material. In the egg shown in Figs. 9 and 10 there are several imperfect spindles and two small but healthy nuclei. One might be somewhat more optimistic about the ultimate fate of cases of this sort, but, since these cases show about the maximum of development in the present material, such optimism is scarcely warranted.

Fig. 11 is introduced especially to show an unusually fine mitotic figure in one of several cells in an egg in which three other cells show more or less normal nuclei. This egg occurred in a follicle in a very advanced stage of atresia and it is rather surprising to find so pronounced a spark of life in a structure so nearly dead.

In all of the cases cited and in nearly all in which similar phenomena were observed the zona pelucida was dense and quite intact, hence there can have been no invasions of stroma or follicular cells. So all nuclei found must be products of the division of the original germinal vesicle. In more advanced atresia, however, the zona begins to open up cracks which admit hordes of stroma cells and leucocytes that feed upon the disintegrating egg material and form cell masses that have been interpreted by some authors as the products of embryonic development. In such cases the observer might be inclined to interpret such synthetic structures, which frequently have an epithelial structure, as tissues, equivalent to those formed in normal embryonic development. Leo Loeb ('11 and '12) in recent papers interprets as placental tissues certain structures

found under similar conditions in the guinea-pig ovary. Not having seen his preparations, but simply judging by his microphotographic illustrations, I am inclined to think that, since he gets no intermediate or advanced cleavage stages, the cell complexes he discusses are open to the interpretation just given for similar phenomena in the armadillo, about whose synthetic origin there appears to be no doubt. Parthenogenetic development in all probability goes no farther in the armadillo than the stages illustrated in the figures. That true parthenogenesis begins and proceeds for a few steps seems assured, but it seems highly improbable, *a priori*, that development could long continue in an environment so unfavorable as that afforded by follicles in which atresia has made such progress as we have seen.

Discussion of the Literature on Parthenogenesis in Mammals.

In 1900 Bonnet in an able paper entitled "Giebt es bei Wirbeltieren Parthenogenesis?" reviewed all the literature dealing with parthenogenesis in vertebrates and gave much space to the question of parthenogenesis in mammals. He concluded that there was no incontrovertible evidence of this mode of development in any of the contributions dealing with the changes described as occurring in ovarian ova during follicular atresia. A considerable number of authors had described ovarian ova containing centrally situated mitotic figures which they had interpreted variously as cleavage spindles or as belated maturation figures. Multicellular masses within zona pelucida were described, in which some cells contained nuclei and others were without nuclei. Some writers considered this condition as a result of a degenerative fragmentation of nucleus and cytoplasm, and others were convinced that the cell mass was the product of parthenogenetic cleavage. Another class of writers called attention to the existence of various kinds of more or less complex ovarian dermoids, epithelioma and other teratoma, which by some were interpreted as the end product of the parthenogenetic development of ovarian ova. Judicially examining all of the evidence before him Bonnet concludes that all of the mitotic figures seen in ovarian ova are to be considered not as

cleavage mitoses but as more or less abnormal maturation figures, that the so-called multicellular embryos are degenerative products, and that teratoma and other kindred phenomena must be explained in some other way than as the products of parthenogenetic cleavage of ovarian ova. This conclusion is, I believe, fully justified by the evidence then available.

Since this review and judgment of Bonnet several authors have reopened the question but have failed to agree. Spuler (1900) insists that the figures which he finds located in the center of ovarian ovocytes, after the extrusion of one polar body, are cleavage spindles, because normally ovulation occurs before this stage and the ovocytes do not develop thus far unless fertilized. He places especial emphasis on finding one ovum in which a centrally located spindle occurred in an egg that had two polar bodies. This, however, is probably a case similar to that described by the present writer (Newman, '12) as due to a precocious division of the first polar body, the spindle being merely the second maturation spindle. Spuler fails to add any material strength to the affirmative side of the question reviewed by Bonnet.

Van der Strict (1901) working with the bat ovary also took the affirmative side of the question as the result of his discovery that ovocytes of the second order occasionally divided mitotically into two cells of approximately equal size. This division could not, he thought, be considered as an anomalous polar body formation, nor as a mitotic division due to degeneration, nor as fragmentation, but only as the beginning of parthenogenetic division. I am inclined to think that Van der Strict was dealing with phenomena closely akin to those described in the present paper for the armadillo, but he failed to make his evidence especially convincing.

L. Loeb (1901) takes a position similar to that assumed by Spuler and Van der Strict and goes a step further in that he holds that the subsequent fragmentation of the egg material into nucleated and enucleated pieces is a progressive phenomenon akin to the parthenogenetic development of the egg of *Chætopterus* which, as Lillie has shown, develops into a larva of considerable complexity without any nuclear cleavage. Loeb figures

one multicellular mass in which a single cell is dividing mitotically, but the spindle is of the size and character of a polar spindle and lacks astral rays. Under the circumstances such a spindle would probably be capable of interpretation as the first polar spindle of an egg that had undergone degenerative fragmentation.

Rubaschkin (1906), as the result of his studies of the guinea-pig ovary, takes a decidedly negative stand, maintaining that all mitotic figures occurring in ovarian ova during follicular atresia are more or less distorted or otherwise abnormal maturation mitoses, and in no sense the mitoses of embryonic cleavage. Multinuclear and apparent multicellular conditions are interpreted by him as the result of degenerative changes brought about by the conditions incident to follicular atresia.

Athias (1908 and 1909) once more reviews the whole situation and presents further data derived from the study of a number of species of mammals. After summing up the evidence pro and con he finds himself unable to reach a definite conclusion though leaning decidedly to the negative point of view. Pending an extensive program of investigation on the subject he prefers to reserve judgment.

My own investigations herewith presented were begun, as stated, in 1909 and, as the reader will have discovered, support the view that a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions.

SUMMARY.

The evidence derived from a study of large numbers of armadillo ovaries demonstrates, I believe, that parthenogenetic cleavage takes place in atretic follicles of this species of mammal. Whether cleavage is preceded by maturation is not clear, but I am inclined to believe that no polar bodies are extruded in those ovocytes that are destined to undergo cleavage.

There is strong reason to believe that the abstriction of the deutoplasmic material in these ovarian ova is equivalent to one of the steps in normal development, for a similar process occurs in the development of several marsupials, the ovogenesis of which shows the same history of deutoplasm formation and reorganization as that seen in the armadillo.

There is no evidence that cleavage proceeds beyond the eight-cell stage, and even at that period there are many signs of advancing degenerative processes. It seems certain, therefore, that, in the armadillo at least, no complex tissue masses such as ovarian teratoma or epitheliomata result from a continuation of the process of parthenogenetic cleavage.

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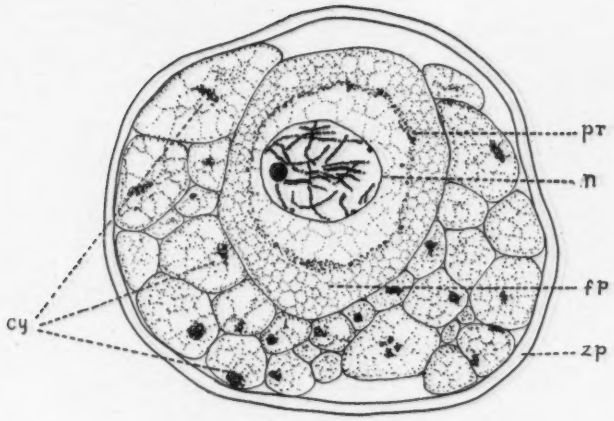
HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April 8, 1913.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Egg with large resting nucleus, showing resumption of chemical interaction between nucleus and cytoplasm preparatory to the formation of the first cleavage spindle. The deutoplasm has been extruded and has been fixed in the form of numerous cytoids (*cy*) that almost surround the egg. The dense zona peludica (*z.p.*) encloses both egg and cytoids. The egg shows a zone of unmodified formative protoplasm (*f.p.*) and a precipitation ring (*p.r.*), where the changes between nucleus and cytoplasm are most active. Note that each cytoid has a centrally located, deeply staining, mass of granules, which are the deutoplasmic granules of earlier stages and are not to be interpreted as degenerating nuclei. (× 800.)

FIG. 2. Egg in which the deutoplasm has just been abstricted and has not yet undergone fragmentation to any marked extent. Two nuclei (n^1 and n^2) lie side by side giving the appearance of a fertilization stage. It is probable that these are the product of the first maturation division, completed without the extrusion of a polar body. This could readily occur in cases where the maturation spindle occupies a central position. These two nuclei would doubtless form a multipolar spindle like that shown in Figs. 13 and 14 and would probably result in the formation of nests of nuclei, as in Fig. 12. Other labelling as in Fig. 1. (× 800.)



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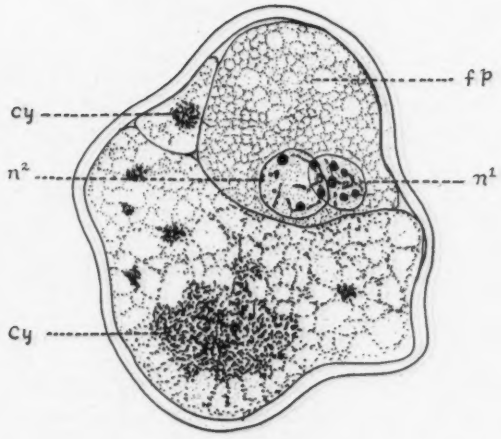

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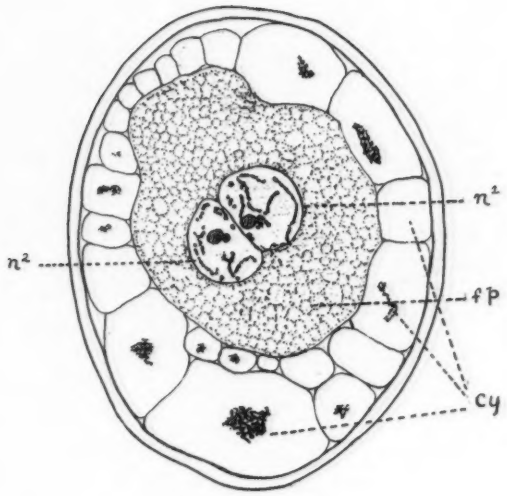
PLATE II.

FIG. 3. Egg with pseudo-epithelium of cytoids and with binucleated egg cell in the center. The explanations given for Figs. 1 and 2 will apply here. (× 800.)

FIG. 4. Egg showing mitotic spindle (*m.s.*) with well-defined polar radiations running from nucleus throughout the cytoplasm. The rather blunt-ended spindle shows mitosis in an anaphase and there are many more than the reduced number of chromosomes, which one invariably finds in maturation spindles. Such a spindle cannot be other than a cleavage spindle. There are only two large cytoids (cy^1 and cy^2). (× 800.)



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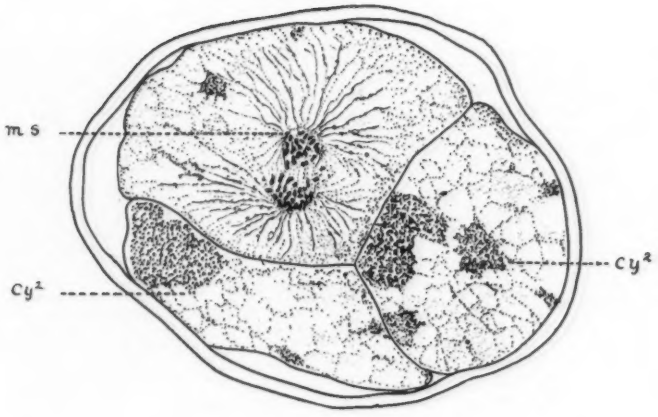

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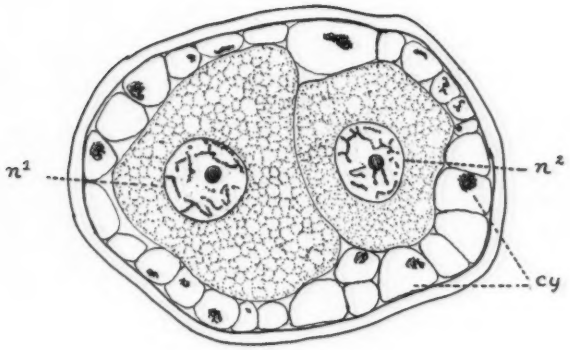
PLATE III.

FIG. 5. A two-cell stage of cleavage. The two cells are of somewhat unequal size, but the nuclei (n^1 and n^2) are both in good condition and in the resting stage. A pseudo-epithelium of deutoplasmic cytoids surrounds the blastomeres. (× 800.)

FIG. 6. A two-cell stage passing into the four-cell condition. Two mitotic spindles are cut transversely to the axis. The spindle on the right ($m.s^2$) is cut near the equatorial plate and shows only a few of the chromosomes. Other chromosomes are scattered through several sections. The spindle on the left ($m.s^1$) is in a late anaphase and has two groups of chromosomes, one at each end of the spindle. The present section cuts through only one of these chromosome groups. Large numbers of cytoids occur in other sections. (× 800.)



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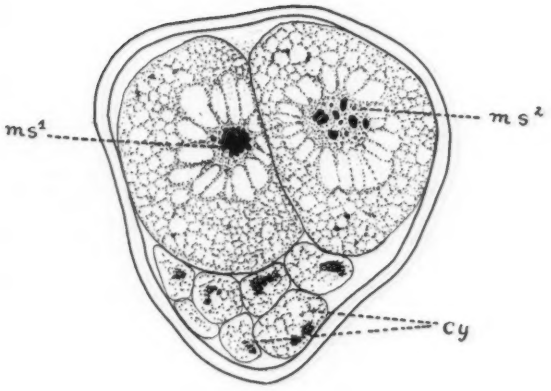

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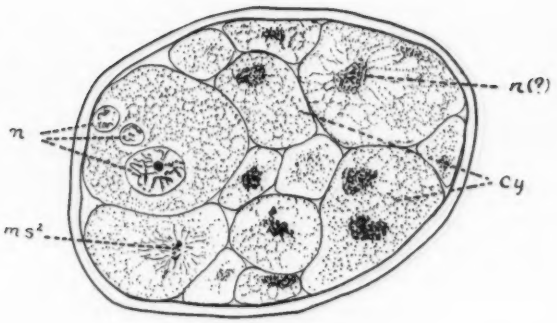
PLATE IV.

FIGS. 7 and 8. Two sections through the same egg, showing one clearly defined mitotic spindle ($m.s^1$), a cell containing three nuclei in the resting stage, one cell with what appears to be a degenerating nucleus (n?), and one cell, shown in both sections, with an imperfect mitotic spindle and only a few chromosomes ($m.s^2$). Cytoids of all sizes are mixed in with the blastomeres. Such an egg seems to be in about a four-cell stage, but will probably not go much further. (× 800.)



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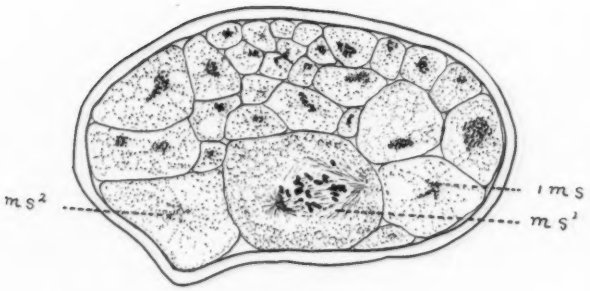

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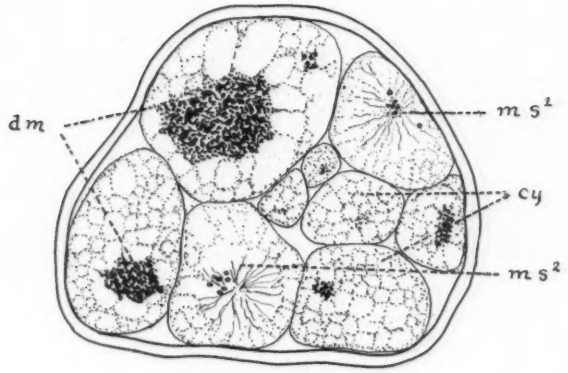
PLATE V.

FIGS. 9 and 10. Two sections through one egg. This seems to represent about an eight-cell stage. There are two cells with resting nuclei (n^1 and n^2) and three cells with more or less perfect mitotic spindles ($m.s^1$–$m.s^5$). Cytoids are scattered among the blastomeres, giving the appearance of cell multiplicity. (× 800.)



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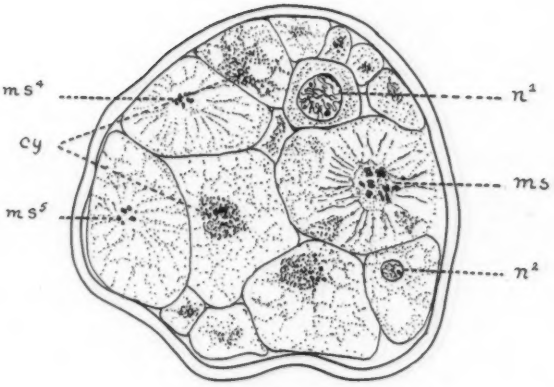

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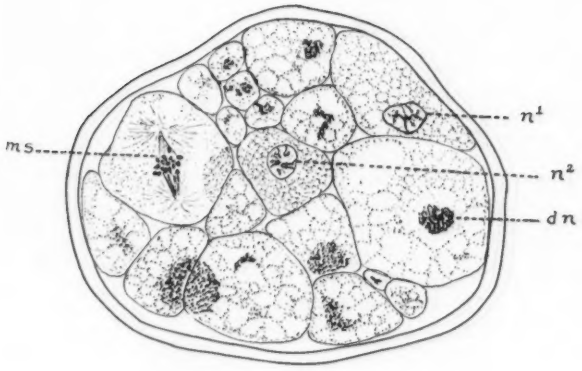
PLATE VI.

FIG. 11. A section through an egg in about a four-cell condition showing the most normal cleavage spindle (*m.s*) found in all the present material. Two other cells show unmistakable resting nuclei (n^1 and n^2). (× 800.)

FIG. 12. An egg with many small nuclei in the formative protoplasm. Such a condition may have arisen through the formation of a multipolar spindle like that shown in Figs. 13 and 14. This is a very frequently found condition. (× 800).



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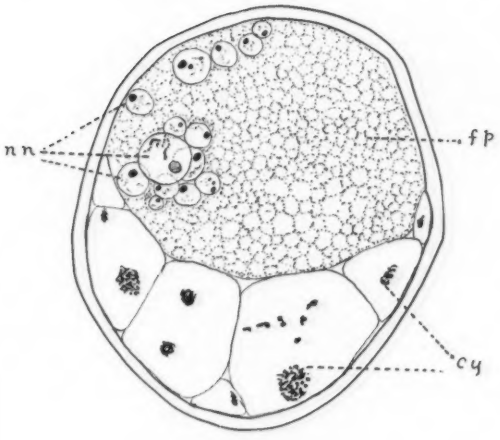

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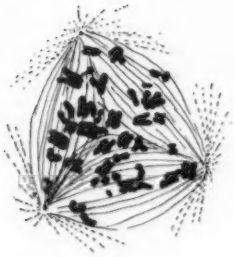
PLATE VII.

FIGS. 13 and 14. Shows the entire chromosome complex in a multipolar spindle which may have arisen from the fusion of two nuclei in eggs like those shown in Figs. 2 and 3. It will readily be seen that the number of chromosomes is very much higher than would arise from a spindle formed from a single nucleus. (× 1,600.)

FIG. 15. An equatorial plate view of a first cleavage spindle of the bipolar type. The number of chromosomes is far larger than the haploid number characteristic of maturation figures. (× 1,600.)

FIG. 16. Another example of the same phenomenon shown in Fig. 15. (×1,600.)



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